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OF

The New York State College of Forestry

AT

SYRACUSE UNIVERSITY

FRANKLIN MOON, Dean

TECHNICAL PUBLICATION NO. 22

THE BIOLOGY OF POLYPORUS GILVUS (SCHW) FRIES

BY

RAY R. HIRT

Instructor of Forest Botany

Published quarterly by the New York State College of Forestry at Syracuse University. Application pending for entry to the second class of mail matter at Syracuse, N. Y. Post Office.

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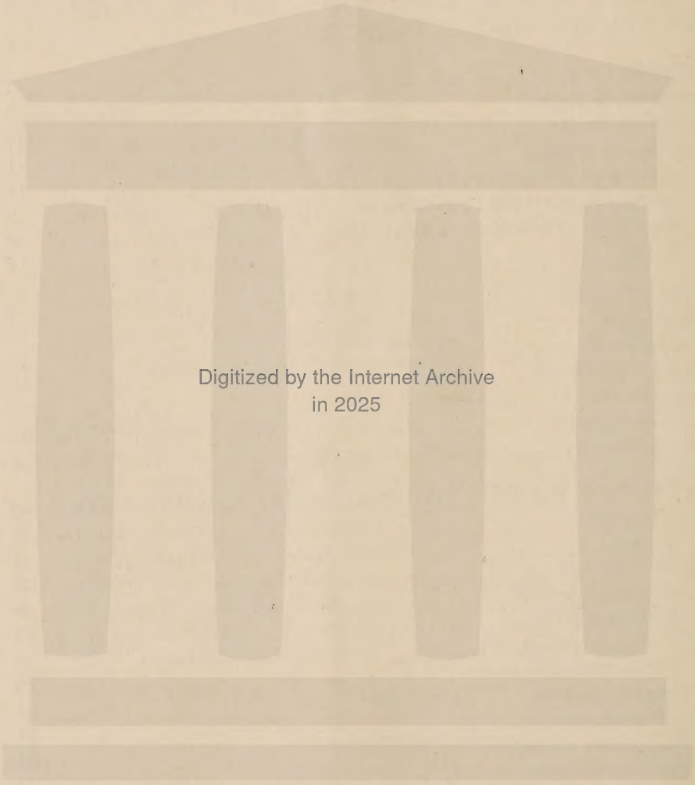

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THE BIOLOGY OF POLYPORUS GILVUS (SCHW.) FRIES [1]

RAY R. HIRT

INTRODUCTION

Among the fungi causing the decay of wood in our forests, the Hymenomycetes are undoubtedly the most important; and of these the Polyporaceae probably cause the greatest amount of damage. In order to combat the inroads of these fungi it is essential that we have certain definite knowledge concerning their habits of growth, spore forms, conditions inciting or inhibiting the growth of the individual fungi, and their effect upon the hosts. With this information available we can take steps to protect our forests to a limited extent from the wood-destroying fungi. Considerable work of this nature has been done but there are still many of the important fungi concerning which very little is known. *Polyporus gilvus*, (Schw.) Fries was among this group. The following work was undertaken with the thought of contributing to our present knowledge of the wood-destroying fungi.

DISTRIBUTION AND HOST WOODS

Among the many polypores attacking timber there are few more widely distributed throughout the world or more abundant within their range than *Polyporus gilvus*. It has been reported as commonly found upon every continent except Europe, where it occurs very rarely and then only in the southern part. In hardwood forests its sporophores may be found upon slash, old stumps, and dead or living tree trunks [2]. Because of its wide distribution and common occurrence it is responsible for the destruction of great quantities of wood annually.

Polyporus gilvus is common throughout the United States and Canada in hardwood forests. The C. G. Lloyd herbarium contains over one hundred collections from the United States and Canada and Mr. Lloyd lists in addition, specimens from Spain, Samoa, Ceylon, Japan, Java, Trinidad, Honduras, New Zealand, Mauritus, Madagascar, Belgian Congo, India, Australia, Isle of St. John, South Africa, Argentine, Brazil, Uruguay, Guatemala, Porto Rico, Mexico, Hawaii, Philippine Islands and Ecuador. Only two European collections are mentioned in literature, one by L. Navas

[1] A thesis submitted to the faculty of The New York State College of Forestry at Syracuse University in partial fulfillment of the requirements for the degree of Doctor of Philosophy, May 1, 1927.

[2] Only twice to the author's knowledge it has been reported growing upon coniferous hosts.

from Spain and the other by Quelet, which is now in the Fries herbarium (26). Its apparent scarcity in Europe is interesting since many of the fungi associated with it in the United States are found abundantly upon the European continent. In the United States the fungus is found from Minnesota on the north to Louisiana on the south, and from the District of Columbia west to Oregon.

The hosts of *Polyporus gilvus* are numerous. Dr. L. O. Overholts states that in Ohio it grows upon dead wood of all kinds (33, 1914). Neuman (32) mentions collections from dead oak, basswood, poplar, maple, hickory, and living trees of oak and maple. Hafiz Kahn (25, 1923) has reported it as an apparent parasite upon *Dalbergia Sisso* Roxb. Rhoads (34, 1918) found it growing upon *Rhus toxicodendron* L. where it was causing a heart-rot within the living stem, an observation of note since but few fungi attack such plants as poison ivy and poison sumach. He (34, 1917) also reports a collection found upon *Tsuga canadensis* (L) Carr. from Pennsylvania. Dr. Weir (46) states that it is not unusual to find it growing upon *Juniperus virginiana* L. in the District of Columbia. *Mangifera indica* Linn., *Liquidambar styraciflua* L., *Rhizophora Manga* L., and *Vitis* species have also been enumerated among its various hosts. The author's collection includes the following host woods: *Quercus alba* L., *Quercus rubra* L., *Castanea dentata* (Marsh.) Borkh., *Fraxinus americana* L., *Hammamelis virginiana* L., *Acer* species, and cultivated varieties of plum, pear and cherry.

In the vicinity of Syracuse *Polyporus gilvus* is found upon *Quercus, Fagus, Prunus, Acer* and *Betula.* Its fruiting bodies occur almost as frequently upon living as upon dead trees. Examination of living trunks showed the fungus to be causing decay in the heartwood or to be decaying necrotic sapwood within a wound. In other words, it apparently attacks woody tissue which consists wholly of dead elements. This fact raised the question as to whether the fungus might ever function as a parasite; namely, whether it invades and causes the death of living cells, a phase of this investigation which is discussed subsequently in a section dealing with parasitism.

History and Synonomy

Polyporus gilvus was first described and named in 1882 by Schweinitz as *Boletus gilvus* from an American specimen. Fries having popularized the term *Polyporus*, described the fungus under that generic name, by which it has since been known. In 1904 Murrill (31, 1908) transferred it to *Hapalopilus gilvus*, but this appellation has not come into common use, the term *Polyporus* being preferred. Sowerby pictured the fungus (Sowerby, t. 195) but Lloyd (26) felt that the illustration was probably that of *Fomes conchatus* (Pers.) Gill. Because of the wide distribution

of the fungus it assumes various forms, many of which have been described as new species as the following list of synonyms indicates.

Murrill records the following:

Boletus gilvus Schw. Schr. Nat. Ges. Leipzig 1:96. 1882.
Polyporus gilvus Fries, Elench. Fung. 1828.
(?) *Polyporus calvescens* Berk. Ann. Nat. Hist. 3:390. 1893.
Polyporus omalopilus Mont. Pl. Cell. Cuba 423. 1842
(?) *Polyporus endozonus* Fries, N. v. Symb. 51. 1851.
Polyporus carneofulvus Berk. Fries, Nov. Symb. 68. 1851.
(?) *Trametes Petersii* Berk. and Curt. Grevillea 1:66. 1872.
Polyporus breviporus Cooke, Grevillea 12:7. 1883.
Polyporus purpureofuscus Cooke, Grevillea 15:24. 1886.
Polyporus aureomarginatus P. Henn. Bot. Jahrb. 22:72. 1875.
Hapalopilus gilvus (Schw.) Murrill. Bull. Torrey Club. 31:418.

Bresadola (9) enumerates several additional synonyms:

Polyporus rubiginosus Berk.
Polyporus Laurencii Berk.
Polyporus cupreus Berk.
Polyporus balansae Spreg.

To this list may be added the forms given by Lloyd (26) concerning which he says, "All the 'forms' have the same 'structure', spores, setae, context, color, etc., but differ slightly in external features. The following nine species are in reality but forms of *Polyporus gilvus.*" .

Polyporus scruposus Fries.
Polyporus inamoenus Mont.
Polyporus Hookerii Lloyd.
Polyporus spurcus Leveille.
Polyporus illicicola Henn.
Polyporus marcuccianus Lloyd.
Polyporus licnoides Mont.
Polyporus auriculiformis Leveille.

Sporophore

Description of Sporophore.— *Pileus* yellow-ferruginous, slightly zonate, somewhat pubescent when young, glabrous upon maturing, roughened, margin thin. *Context* yellowish brown, soft corky when fresh, to woody when dry. *Pores* small, round to angular, 5–8 per mm. *Tubes* 0.5 to 12 mm. long, walls gray to light brown, mouths a faded brown; *setae* averaging 3–8 in cross section of tube, brown, tapering to a point, projecting 12–16 μ into tubes. *Spores* white, smooth, oblong-ellipsoid, 4–6 x 3–4 μ, containing a large globule.

A section through the sporophore from the base to the margin shows darkened bands throughout the context corresponding to the zonations on the upper surface. If a smooth cut be made, the exposed surface has a distinct sheen. The hyphae grow out-

ward perpendicular to the substratum, bending down sharply to form the tube layer.

In direct frontal view the pore surface is a deep brown but as the sporophore is turned obliquely, the color appears light brown and at times almost gray. This phenomenon is traceable to the fact that the tubes are irregular in outline. One side of the tube is longer than the other, and when the pore surface is viewed obliquely, on the one hand the faded-brown hymenium is visible while the opposite view presents the deep brown mouths of the tubes.

The fruiting bodies vary both in size and shape, depending upon their position on the host, the moisture supply, and the temperature. The largest single specimen examined was 9½ x 4 cm. in length and breadth respectively; the smallest 3 x 2 mm., the latter being a mature sporophore growing among several larger ones. The thickness varies from 1 mm. to 3 cm. The sporophores may be found singly or imbricated, and shelving or resupinate, depending upon their position on the substratum. The variation in form is so great that, since *Polyporus gilvus* was first described by Schweinitz, it has been named and renamed no less than twenty different times and assigned by various workers to *Polyporus, Trametes, Fomes,* and *Polystictus.* This diversity in form is particularly noticeable in tropical specimens. While the thickness of the pileus, the length of the tubes, and the appearance of the upper surface vary somewhat, nevertheless the color of the context, the shape and size of the spores, and the presence of the sharply pointed setae are characters sufficiently constant to permit the ready identification of the species.

Stratified Sporophorus[1].— During the course of this work many sporophores were examined which showed distinct stratification of the tubes. In his description of *Polyporus gilvus,* Dr. L. O. Overholts reports that "the plants are annual or reviving for two or three years" (33, 1914) and further that "the plants are sometimes perennial" (33, 1915). The generally accepted basis for the separation of the genus *Fomes* from *Polyporus* is the habit of perennial growth, whereby a new layer of tubes is added to the sporophore annually by the viable hyphae of the context or tube layer.

During the summer of 1923 a study was made of the development of some sporophores of *Polyporus gilvus* growing under natural conditions in the vicinity of Syracuse. In these fruiting bodies, at least, stratification of the tube layers did not indicate perennial growth.

In February 1924, a section from the trunk of a white oak infected with the fungus was brought into the laboratory. Upon this were a few sporophores which had been formed during the summer of 1923. Several of these were split to make sure that there was

[1] Hirt, R. R. Stratified Sporophores of Polyporus gilvus. Mycologia, 28:111–113. May–June, 1926.

only one layer of tubes present and they were left attached to the bark. The remainder were left intact. Tissue cultures from these were attempted but no mycelial growth was evident, although cultures from decayed wood were easily obtained.

Within forty hours after the wood was brought into the laboratory small patches of mycelium were noticed around the bases of some of the sporophores of 1923. Tissue cultures were again attempted from these sporophores without result, although cultures from the patches of mycelium developed rapidly. It is manifest that the hyphae of the context and tube layers of the 1923 sporophores were dead and not capable of further growth. The fresh mycelium which formed the above mentioned patches, was at first white and fluffy but soon became compact and brownish. Gradually it spread outward, using the dead sporophores as a substratum upon which to grow and eventually wholly enveloped them, covering the upper surface with a new context and developing a new layer of tubes upon the under surface from which spores were cast. In general, the new growth on the upper and lower surfaces of the old sporophores kept pace with each other so that they met at the margin, fused, and continued to develop as a single body from that point. At this stage of development, one of these compound pseudo-perennial sporophores was removed and tissue cultures were again attempted from the enclosed sporophore, as well as from the one enclosing it. From the former no growth was obtained; from the latter, cultures were produced, again proving conclusively that the sporophore of the previous season had no active part in the production of the new context and tube layer.

The remaining sporophores about which new growth had appeared were allowed to develop until late May when they were removed and sectioned. Each one of these exhibited pseudo-perennial stratification similar to that which had been observed in the field, and traceable to the engulfing of the fruit bodies of the previous year.

Late in the summer of 1924 sporophores of the current year upon a white oak and a white ash were marked for observation during the following summer. Early in June 1925, new patches of mycelium appeared at the bases of several of these fruiting bodies. They were carefully watched and were found to develop similarly to the above, so that in late summer when the sporophores were gathered and sectioned, they might have been mistaken for perennials had their formation not been observed.

A section of a stratified sporophore taken from the base outward through the margin, shows that a very thin layer of mycelium, 0.1 to 0.5 mm. in thickness and similar to the mycelium of the context, is formed at the base of the first tube layer. These hyphae are compact and firmly interwoven and extend a short distance

upward into the tubes of the older sporophores, while others bend sharply downward to form the new tube layer. In peeling away the enveloping sporophores, this layer of compact hyphae remains attached to the younger tubes. The new growth over the older context is much thicker, varying greatly with the different conditions affecting its development, such as temperature and moisture supply. It has all the appearance of the first formed context in texture, color, and darkened bands, but is less firmly attached to the old context than the new tube layer is to its substratum.

The evidence at hand indicates that the mycelium of *Polyporus gilvus* lives in decayed wood from year to year and is therefore perennial. It also proves that the stratified sporophores of the fungus developed in this section of New York State are annual, and suggests the same might hold for other sections of the country if careful studies of the developing sporophores were made.

Reaction to Gravity.— The sporophores are distinctly geotropic in their development; that is, the tubes are always aligned parallel with the direction of gravity. If the position of the sporophore is changed while actively growing, a corresponding change in the "growth direction" of the tubes results. A number of experiments were made to test this reaction.

A sporophore partly enveloped by a new hyphal growth was found on a fallen branch. Spores were being cast from the new tubes, and the upper surface of the old sporophore was partly covered with a new context. The branch was turned completely over so that the pore surface pointed upward and the context downward. After several days tubes began to develop upon what originally had been the upper side of the context. At the same time over the original pore surface warty growths appeared, and developed into many small sporophores which eventually covered the old pore surface and produced tubes parallel to the original tubes, but with the direction of growth diametrically opposite.

Similar experiments were tried with fruiting bodies under observation in the laboratory with corresponding results. When the fruiting bodies were turned over, the original context always developed a tube layer with the mouths opening downward, but the first formed layer of tubes occasionally failed to produce a new context over the tube mouths. This resulted in a sporophore with tubes opening both upward and downward, although spores were cast only from the latter.

Growing sporophores were next revolved ninety degrees from the original position. The enlargement of the sporophores ceased, but new sporophores developed from the hymenophore at right angles to the first pore surface. These results agree with Buller's (10) conclusions, that the growth of the sporophore proceeds in such a manner as to orientate the hymenium so that the spores may fall freely downward through a tube from the mouth of which they may be carried off by the wind.

Production of Spores.— Spores have been gathered in the field as early as July 2 and subsequently until the second week of August, depending upon the season. The spore-casting period of a given sporophore, however, appears much shorter. From a single specimen in the field, spores were never gathered in any quantity for more than three successive nights. In the laboratory, to the contrary, single sporophores have cast spores for twenty-four consecutive nights, in fact, until the experiment was terminated when a new layer of tubes began to form over the old pore surface and to cast spores. Possibly, under optimum growing conditions in the field, spores might be cast over equally long periods of time from a single sporophore.

Spores are not produced immediately upon the formation of pores. Cross sections of newly formed tubes contain hyphae which grow centripetally into the openings and are densely covered with crystals. No spore-bearing basidia are to be seen while these crystal-bearing hyphae are present. As the tubes gradually lengthen, the crystal-covered hyphae disappear and basidia which bear spores develop.

The casting of spores is a continuous process, although sporulation is more heavy at certain periods than at others. During the day sufficient spores may be cast to give a misty appearance to a glass slide. During a similar period at night, in comparison, spores may be set free in numbers sufficient to give the slide the appearance of being covered with a white powder. The heavy nocturnal discharge of spores was so noticeable that it seemed desirable to experiment further to bring about, if possible, a change in the spore casting intervals.

Sections of oak trunks varying from eight inches to two feet in length, which were partly decayed by *Polyporus gilvus,* were brought into the laboratory. Sporophores had already developed upon some, while it was hoped that fruiting bodies would form on the others. The blocks were placed on end in pans of water in a room where the temperature could be regulated. The previously formed sporophores continued to grow and cast spores, and new sporophorus developed within eight days on the other blocks. Spore traps were placed beneath each sporophore. The first experiments covered a period of twenty-three days. Later experiments were carried on for varying periods over two years.

Sporulation as Related to Light.—(a) One group of sporophores was kept at approximately the same temperature both day and night, namely 25°C, with natural daylight and dark periods. The moisture conditions remained the same.

TABLE 1.—*Sporophores kept under normal daylight and dark periods.*

	8 A. M.—8 P. M.	8 P. M.—8 A. M.
C.	Daylight	Darkness
25°	Light sporulation	Heavy sporulation

Spore casting was exceedingly heavy at night and very light during the day. In the experiment, night was considered as extending from 8 P. M. to 8 A. M., and day from 8 A. M. to 8 P. M.

(b) The second group was subjected to light and dark periods which were just the reverse of group one. During the day cultures were placed in a dark chamber and at night they were left in the laboratory with two electric light bulbs giving a strong light.

TABLE 2.—*Light and dark periods the reverse of those in table 1.*

	8 A. M.—8 P. M.	8 P. M.—8 A. M.
C.	Dark chamber	Artificial light
25°	Light sporulation	Heavy sporulation

The interval of heavy sporulation remained the same as in the former group. Since the temperature and moisture remained the same, and the change in light and dark periods did not alter the periodic casting of spores, it is very evident that the absence of light was not the stimulus for the heavy noctural discharge of spores.

Sporulation as Related to Temperature.—Although the previous experiments indicated that light did not affect periodic sporulation, it was decided to test for light reactions while varying the temperature. As Table 3 shows, light again proved to be a negligible factor. Temperature (within the range favorable for spore production) however, while not affecting the periodic sporulation, did act as a controlling factor in the number of spores cast during both light and dark periods.

TABLE 3.— *Showing the effect of temperature changes upon sporulation*

	8 A. M.— 8 P. M.		8 P. M.— 8 A. M.	
C.	Daylight	Dark chamber	Artificial light	Darkness
33°.....	Very light sporulation	Very light sporulation	Light sporulation	Light sporulation
31°.....	Very light sporulation	Very light sporulation	Light sporulation	Light sporulation
30°.....	Light sporulation	Light sporulation	Heavy sporulation	Heavy sporulation
27°.....	Light sporulation	Light sporulation	Heavy sporulation	Heavy sporulation
25°.....	Light sporulation	Light sporulation	Very heavy sporulation	Very heavy sporulation
22°.....	Light sporulation	Light sporulation	Heavy sporulation	Heavy sporulation
20°.....	Very light sporulation	Very light sporulation	Light sporulation	Light sporulation
19°.....	Very light sporulation	Very light sporulation	Light sporulation	Light sporulation
16°.....	Very light sporulation	Very light sporulation	Very light sporulation	Very light sporulation

From 8 P. M. to 8 A. M. the spore deposits on the slides were approximately the same between the temperature of 22° C. and 30° C. The heaviest discharge of spores occurred within range of a degree or two above or below 25° C. Above 30° C. and below 22° C. a decrease in the number of spores was clearly evident. Above 33° C. and below 19° C. an exceedingly light deposit of spores occurred. For the same changes in temperature, corresponding changes in the numbers of spores cast took place during the hours from 8 A. M. to 8 P. M.

Throughout the experiments there were occasional heavy spore casts during the daylight hours and light sporulation at night. The tendency, however, was toward a distinct periodic casting of spores, heavy at night and light during the day, regardless of temperature changes within the limits of temperatures favorable for the casting of spores.

Sporulation as Related to Moisture.— Moisture was an important factor in sporulation. If the water level in the pans lowered to the extent that moisture decreased noticeably in the region of the sporophore, no spores were cast. If electric bulbs were placed sufficiently close to a sporophore so as to cause moisture to evaporate rapidly enough to give the sporophore the appearance of drying, sporulation ceased. In this case the temperature would also be raised which in turn would affect the spore producion. No young sporophores were ever produced on these blocks above the

region where, to the unaided eye, it was evident the wood was not very moist.

Atmospheric moisture was not a controlling factor in the above experiments since the amount of moisture remained the same during daylight and dark periods.

With moisture and temperature conditions favorable, the sporulation was more prolific from 8 P. M. to 8 A. M. than at any other period during the twenty-four hours. Light was proven to be no factor in this periodic tendency and no other cause was evident.

Spores.— En masse the basidiospores are white. Viewed under the microscope they are hyaline, oblong-ellipsoid, 4–6 x 3–4 μ, with a distinct apiculus and large globule. (Plate VII, Fig. 13.) In sections taken through the tubes of *Polyporus gilvus,* it is evident that the spores are attached at the apicular end to the slightly curved sterigmata, which are approximately the length of the mature basidiospores.

The sterigmata reach their full length prior to the development of the spores. At first the spores appear as swollen globular tips of the sterigmata (Plate 9, Figures 17 and 19) but as they mature, gradually assume their typical shape at which time the large globule appears. This globule does not stain with safranin, gentian-violet, Haidenhain's iron-alum haematoxylin, or alcannin. In one end of each spore a small body stained with each of these stains except alcannin, and this probably is a nucleus, although it could not be positively determined as such even when observed under an oil immersion lens. The apex or outer end of each spore is more convex than the basal end, just as the outer side is more convex than the inner side (Plate 9, Figure 18).

CULTURE STUDIES

Source of Cultures.— Cultures of *Polyporus gilvus* were obtained from several sources:

(1) Tissue cultures were made from fresh sporophores, but with little success since *Penicillium* was abundant upon the fruiting bodies. In spite of the greatest care a large proportion of the cultures became contaminated with *Penicillium.*

(2) Decaying sapwood or heartwood, either in extreme or incipient decay, proved to be a satisfactory source from which to obtain cultures. If wood was taken some distance back from the bark, fungal contamination was seldom encountered.

(3) Fresh spores were obtained by means of spore traps from fruiting bodies growing both in the field and in the laboratory. The spore traps consisted of glass slides of the usual type. They were sterilized and placed upon large pins pushed into the wood directly beneath the sporophores. At times the fruiting bodies and slides were covered with heavy paper boxes which had likewise been sterilized, usually, however, the traps remained uncovered. The spore-covered slides were stored in sterilized Petri dishes.

The appearance of the mycelium on the culture medium was the

same regardless of the source of the culture. Tissue cultures made from actively growing sporophores developed much more rapidly than those from any other source.

In passing it should be pointed out that, when pure cultures were desired, they were invariably started from a single spore in accordance with Palladin's definition of such cultures. A pure culture (Palladin's Plant Physiology, page 56) is one which contains only a single definite species of organism and can be obtained only by fulfilling two conditions. The first consists in exercising sufficient precaution to prevent contamination of the sterilized culture medium; the second in the derivation of the culture from a single cell, in this instance a basidiospore.

Culture Media.—Spores were germinated upon various culture media such as: sterilized potato, carrot, string beans, wood decoctions in agar, synthetic agars, blocks of deciduous and coniferous woods, and in distilled water. Abundant vegetative growth was obtained upon Leonian's agar, although fruiting bodies never developed upon it. In general the best results were obtained upon Duggar's (14) nutrient agar prepared as follows:

Fifteen grams of agar were cut into small pieces and added to 900 cc. of distilled water. This was slowly heated and stirred until the agar was thoroughly dissolved. To this was added

1.0 gram ammonium nitrate.
.5 gram dihydrogen potassium phosphate.
.25 gram magesium sulfate.
trace of iron chloride.
5.0 grams cane sugar.
100.0 cc. distilled water.

The solution was filtered and tubed into 55 cc. culture tubes which had previously been dry sterilized. The tubes of agar were put into an autoclave for twenty minutes under eighteen pounds of steam pressure, after which they were placed in a position so that agar slants were formed.

Spore Germination.—Spores for the first cultures were obtained from deposits on the spore traps placed out in the woods. The high percentage of bacterial and *Penicillium* infection from this source was quite disconcerting. A very large number of cultures had to be started in order to secure any free from contamination. The period of obtaining spores was usually quite short due to changing atmospheric conditions, and came at a time when it was not always convenient to carry on culture studies. Sporophores could not be induced to revive in the laboratory. A method was discovered whereby fresh sporophores might be grown and fresh spores obtained at most any season of the year they were desired.

During the summer months logs, stumps, branches, and dead trunks infected with *Polyporous gilvus* were located. When fresh spores were desired during the winter months, blocks of infected wood from these sources were brought into the laboratory. The blocks were placed in pans which contained a small amount of water. They were kept in a room with an average temperature of

22° C. Within forty-eight hours fruiting bodies usually developed above the water line, and spores were soon produced. It was then a simple matter to arrange a spore trap and collect quantities of spores which were quite free from spores of foreign fungi.

It so happened that no blocks were brought into the laboratory during the late fall, but from January 1 to September 15, this method was used successfully. *Trametes suavolens* (L) Winter responded similarly but much more slowly, and it is possible that other fungi might be induced to fruit by the same method.

Van Tieghem cells were used for germination tests but with only partial success. Spores were placed upon hardened agar within the cell; they were mixed with liquid media; stirred into distilled water and tap water; placed dry on the cover-glass and then covered with agar. But at best the percentage of germination was low if it took place at all. A better method for germinating spores was found in the use of Petri dishes, in which spores from the same sources as those used in the Van Tieghem cells, showed a reasonably high per centage of germination.

Petri dishes were carefully sterilized and then partially filled with the culture medium. Spores were introduced into a small amount of sterilized water, loopfulls of which were placed upon the surface of the agar in the Petri dishes. Water, which had condensed on the inner surface of the Petri dish covers, was shaken down and washed over the surface of the inoculated agar. In this way the spores were washed about and separated sufficiently to enable one to be removed with a stab when desired for a single spore culture. The bottoms of the dishes were marked on the outside so that individual spores could be definitely located and readily found again. With care, the cover of the dish could be removed and the germination of the spores watched under the microscope. Ordinarily the cultures remained free from contamination for many days.

It was found that spores taken from certain traps would not germinate under any conditions tried, whereas spores from other traps gave practically one hundred per cent germination. White (45) found the spores of *Fomes applanatus* (Pers.) Wallr. to behave similarly. No explanation could be given other than the fact that some sporophores evidently produced non-viable spores.

Germination was carried on at ordinary room temperature. Light or darkness had no apparent effect upon the rate or percentage of germination. The percentage of germination was never uniform, but varied from practically one hundred per cent with some spores to no germination whatsoever with others. Germination occurred from twenty to thirty hours after placing the spores upon agar in covered Petri dishes.

The first evidence of germination was a slight swelling, followed by the appearance of a germ tube, which was invariably put out at the apex of the spore, that is, the end opposite the apiculus. As it increased in length the globule in the spore decreased in size. A small globule often appeared in the germ tube and usually

assumed a position near the anterior end of the tube. A single spore was never observed to put out more than one germ tube, although occasionally the tube might branch at right angles to itself almost immediately after appearing. Spores germinating close together influenced the direction of growth of the germ tubes. The young hyphae would turn aside from their initial direction of growth as they approached another hypha or spore (Plate 10, Figure 20). It was not unusual for a germ tube to branch when approaching another spore and for the branches to grow around either side of it. When spores germinated close together the young mycelium was always very much branched and twisted in its growth, in avoiding other germ tubes and spores. If the spores germinated at some distance from one another, the germ tubes and young mycelium tended to grow in one general direction, and ordinarily did not branch so early nor so profusely.

Vegetative Mycelium.— The germ tube developed directly into what might be termed the primary mycelium. The hyphae were approximately the width of the spore; septations were few; no clamp connections nor anastomosing of hyphae occurred. Branching took place within forty-eight hours, the branches retaining the width of the original hypha. Within ninety-six hours the secondary mycelium began to develop. Branches from the primary hyphae became distinctly narrower, septations increased slightly in number but never became numerous; branching took place differently. In the primary mycelium branches appeared as though budding from a hypha (Plate 8, Figure 15). In the secondary mycelium the tip of a hypha would swing aside from its initial direction of growth, and at the bend thus formed, a branch would later occur (Plate 8, Figure 16). Such branching is frequently noticed within cells of wood and in the context of sporophores. Anastomosing took place infrequently, but clamp connections were never found.

Hafiz Kahn (25, 1925) found hyphae which "were hyaline or pale colored, septate and provided with clamp connections" in wood of *Dalbergia Sisso* attacked by *Polyporus gilvus*. He stated that by isolating this mycelium and inoculating sterilized decayed wood, he found that sporophores of *Polyporus gilvus* developed. During five years of work with the fungus in artificial culture and in nature, the author never found any evidence of clamp connections in the hyphae of the fruiting bodies, on agar, or in cells where hyphae were noted. Examinations of the mycelium were made with an oil immersion objective wherever possible. These hyphae which lacked clamp connections also produced fruiting bodies of *Polyporus gilvus* both on nutrient agar and wood blocks.

No secondary spores were ever produced in culture.

Spore Cultures.— Spores for this work were obtained in the same manner as for the germination tests. When spores were beginning to germinate on agar in the Petri dishes, those desired were removed with a stab and placed in individual dishes of agar. Their development was observed for a time until it was determined

positively that no foreign spores were present, after which they were transferred to tubes of agar.

Several days after inoculating an agar-slant, a loose, fluffy, white mycelium appeared, spreading out in all directions from the point of inoculation. The hyphae gradually became more compact and appressed to the agar surface. At intervals on this surface clumps of white, powdery-appearing hyphae were evident. Upon examination they were found to be covered with minute crystals, similar, possibly to those White (45) found to be present in cultures of *Fomes applanatus*. The crystals dissolved in hydrochloric acid without effervescing. Following the appearance of these white clumps, the areas began to show a tinge of clay color (36). Gradually these spots became less and less powdery in appearance and more brown. The hyphae became very compact and abnormal sporophores developed. These fruiting bodies bore viable spores from which new cultures were made and carried on through several generations of the fungus.

The first formed fruiting bodies were irpiciform in appearance, bearing basidia along the sides of the tooth-like projections from which spores were cast. The sporophores formed later in these cultures and others, produced true tubes and pore surfaces typical of those found in nature. No truly shelving types were formed on agar. Sporophores were produced in culture when exposed to both direct and diffused light. Light struck the cultures from all sides, whereas Long and Harsch (27) in their work found that fruiting bodies of fungi on artificial media were obtained when light struck the tubes in a line parallel to the tubes placed in an upright position.

From the planting of the spores to the production of new spores by young fruiting bodies the minimum time was 14 days; the maximum was 105 days. Tissue cultures taken from these tubes produced fruiting bodies within 13 days after inoculation.

A number of pure cultures in Petri dishes produced an abnormal hymenium with no indication of a fruiting body, either in the form of colored hyphae or a compact mass of mycelium. Shortly after the appearance of the crystal-covered hyphae, basidia appeared upon them. The surprising feature was that occasional basidia produced five basidiospores (Plate 9, Figure 19). The fact that the mycelium was growing upon the flat upper surface of the agar, and therefore had no opportunity to form tubes in the normal position, might be offered as a partial explanation for the abnormal occurrence. When the dishes were turned over so that the agar surface was downward, fruiting bodies developed at these places and produced a normal hymenium within tubes.

In the case of tube cultures the bodies were always formed upon the slant in such a position that the tubes were in line with the force of gravity, similar to what Harsch and Long (27) found to be true in their investigations with other fungi. Setae were always produced in these sporophores on artificial media. In the irpiciform type it was possible to trace the setae-producing hyphae

from the part corresponding to the context in normal sporophores, out through the teeth. Some of the setae projected out from between the basidia along the sides of the teeth; others extended through the trama and out at the ends. In those sporophores producing pores, the setae were not so easily traced, as the hyphae of the trama and context were more compact. The deep brown setae arose from hyphae which were greater in diameter and thicker walled than the surrounding hyphae. In the context of normal sporophores similar hyphae may be observed and no doubt they give rise to the setae.

On wood, as well as on agar, single spore cultures were made. The spores were secured, germinated, and isolated as previously described. Pieces of sound and decayed heartwood and sapwood of white oak and white ash were gathered and cut into lengths suitable for placing in culture tubes. The blocks were first soaked in sterilized distilled water and then placed upon glass beads in dry-sterilized tubes. Distilled water was added in sufficient quantity to extend above the base of the blocks. These were autoclaved for one half hour each day for three successive days at eighteen pounds pressure. Controls kept for a period of two years showed that no living organisms remained in the wood after this treatment. When the blocks had cooled after sterilization, a cubic millimeter of agar with a germinated spore was placed upon the upright surface of each. The tubes were then put into small battery jars which had been sterilized with mercuric chloride. Vaseline was smeared over the edges of these jars after which each was covered with a glass plate. These containers were placed in still larger battery jars which were washed and sealed in the same manner. In the latter an open dish of distilled water was set. Cultures kept in this way were still moist at the end of three years and none of them became contaminated with other fungi.

From the inoculum the mycelium spread outward in all directions, covering the upper ends of the blocks. Gradually it grew downward over the sides of the wood; however, here it grew as fiber-like threads in the form of a net in contrast to the mat-like formation on the upper surface. Tawny wart-like masses of mycelium appeared at irregular intervals and later developed into sporophores of *Polyporus gilvus*, which showered the sides of the test tubes with spores, from which new spore cultures were obtained. The fruiting bodies were typical of those found under natural conditions, even to the extent of being distinctly shelving.

Hafiz Kahn (25) states, "It has been repeatedly experienced while working with this fungus (*Polyporus gilvus*) that slides of healthy shisham wood when inoculated with pure cultures have never succeeded in producing artificial sporophores." He used decayed wood of shisham as a culture medium. In the present work no difficulty has been experienced in obtaining fruiting bodies from cultures upon normal wood of ash and oak. In fact, cultures upon partially and extremely decayed wood of both hosts produced a very scanty growth of mycelium and never produced sporophores.

Cultures upon blocks of hard pine kept for two years under the same conditions as cultures on hardwoods, produced a sparse growth of mycelium which was limited to scattered areas on the blocks. Tawny compact masses of mycelium appeared but no sporophores ever developed. Pockets of decayed wood were found beneath the patches of mycelium but they were small in diameter and shallow. Hard pine has never been cited as a host for this fungus but under favorable conditions may serve as such.

Homothallism.— Cultures were started from single spores, others from two to many spores were made. In each case sporophores were produced in about the same period of time. The fruiting bodies were similar in size, shape, and numbers per culture tube, and all produced viable spores. There were no clamp connections found in any of the cultures. Anastomosing was as frequent in monosporus cultures as in those where the mycelium from several spores mingled. Thus *Polyporus gilvus* is a homothallic fungus; that is, not only can the mycelium from a single spore cause decay in a host wood, but from its vegetative growth new fruiting bodies may be produced from which millions of viable spores are cast. In other words, the production of fertile fruiting bodies is not dependent upon the fusion of nuclei from the mycelium of different plants.

Tissue Cultures. — Tissue from live sporophores in the field gave a great amount of contamination, particularly from *Penicillium* It has been experienced that almost invariably this fungus is associated with the live sporophores of *Polyporus gilvus*, and to get a portion of living tissue with no *Penicillium* present was very difficult. Once a pure culture of *Polyporus gilvus* is obtained from this source, it develops to the fruiting stage very rapidly.

From decayed wood, cultures can be readily obtained. Incipient or extreme decay gives equally good results providing the material is taken at some distance back from the exposed surface. Bacteria are seldom present and foreign hyphae are not often encountered.

The Destruction of Wood by Polyporus Gilvus

Decay in General.— Decay is the resultant action of the enzymes secreted by the hyphae of the invading fungus upon the wood elements. These enzymes produce a chemical change whereby certain constituents of the cell contents and cell walls are dissolved, leaving a more or less disintegrated mass of wood elements which break apart and crumble under the application of force. Thus the wood is made unfit for commercial purposes. Such is the effect upon woody tissues attacked by *Polyporus gilvus.*

Resistance to decay by certain filamentous fungi and bacteria is dependent largely upon the chemical nature of the wood, namely the cell walls and cell content. It is not correlated to any great degree with physical properties, such as weight, strength, or structure. The cell contents vary greatly. The protoplast itself is made up of complex compounds which vary in kind and proportion in different plants and different tissues of the same plant.

The protoplast contains in varying amounts protein granules, starch grains, oils, crystals, tannis, etc., some or all of which may serve as food for the invading fungus. The cell wall is composed fundamentally of carbohydrates of the cellulose group. It soon becomes changed by a process known as lignification. Lignification is a complex chemical process, and that which is spoken of as lignin is a group of complex substance concerning which little is known.

By some botanists lignin has been considered as a nitrogenous compound which will give definite color reactions in response to certain dyes (1). Cellulose has also been thought of as responding with definite color reactions to given chemical reagents. The chemists do not wholly agree to this (12) (40). As defined by a chemist, lignin is "certain organic compounds of the cell wall which function in increasing the strength of that wall. These have been grouped together under the collective term lignin" (20) Further, the chemist is able to remove this material which he calls lignin from the cell wall, and he cites examples where stains used by botanists are not true indicators of it nor of the chemical changes going on in the process of decay (40). That is, we have arrived at a period when the results obtained through the use of microchemical stains alone are not sufficient evidence of what occurs during the decay of wood. A chemical analysis should be made as a final check. For this work both means of determining what effect *Polyporus gilvus* has upon the host tissue were employed. How well the two agree will be evident in the discussion which follows.

Field Studies.— *Polyporus gilvus* is ordinarily considered as a sapwood-rotting fungus, however, it is by no means limited to that tissue. It has been reported by Rhoads (34, 1918[1]) as causing a severe heart-rot within a living host, and given favorable conditions for growth, it will work into the heartwood of trees while causing the decay of the sapwood.

The decay associated with the fungus is quite characteristic regardless of the host upon which it is found, or whether it is growing in the sapwood or heartwood. It causes a typical "white rot," the wood becoming lighter in color as decay advances.

Decayed wood is a light cream color, inclined to be brittle when dry, tending to split tangentially along the limits of the annual rings, and radially along the wood rays. It forms a rather clean break in either direction. Any cracks or beetle tunnels are filled with mats of brown hyphae. Throughout the bark enclosing decayed wood, threads of brown hyphae are in evidence. The transition from normal to decayed wood is very abrupt with no black lines of decay visible. In wood two degrees of decay are found, namely, incipient and extreme decay. Incipient decay of wood in common with other rots, is characterized by a change in color, being slightly lighter than the normal wood, and appears as though it had been bleached. The rays are firm and not discolored, standing out sharply against the background of the bleached-out

elements. The resistance to cutting is almost as great as in normal wood because of the apparently unaffected rays. In extreme decay all the elements of the wood have lost the normal color, giving a bleached appearance to the whole area. The rays still stand out sharply since they are darker than the surrounding elements, but they have lost their firmness and crumble as readily as the other parts. The change from the early incipient decay to the most extreme stage is marked by the increasing ease with which the elements can be crumbled, and by the bleaching of the wood rays. The wood becomes very light and dry. Observed through a hand lens the elements appear to be intact, to the extent at least, that the wood can usually be identified when the shape, size, and distribution of the elements are the deciding factors.

Decay of White Ash

Macroscopic Studies.— White ash is rather resistant to the attacks of fungi, nevertheless upon a large percentage of such trees in a nearby woods, sporophores of *Polyporus gilvus* were found. The grass and underbrush had been burned away several years previous, leaving fire scars upon the bases of many trees. It was within these fire scars that the fruiting bodies were noticed. The ash trees were in a mixed stand of white and red oak upon which sporophores of this fungus were abundant. While fruiting bodies of various fungi were found upon the oak trees, only those of *Polyporus gilvus* appeared on the ash. The decay in a number of the ash trees was studied but no apparent differences were detected in the several trees. A tree was selected for detailed study which showed typical decay caused by the fungus in question.

The tree chosen was approximately forty-two years old and presented a healthy appearance except for the presence of sporophores near the base. It was felled and cut into short lengths. The fire scar was seven inches wide at the root collar; five inches wide thirty-seven inches up from the base, and tapered off rapidly within the next few inches. Five feet from the root collar no indication of decay was present. Sections of the tree extending from the base up the trunk six feet were brought into the laboratory for study. Within the fire scar at the root collar, a decayed area four inches wide extended three inches back into the heart of the tree in the form of a semi-circle (Plate 2, Figure 4). Thirty-four inches up the trunk it was two and one-half inches across and two inches deep; thirty-nine inches up the trunk there was no evidence whatsoever of decay, either to the unaided eye or under the microscope. Sporophores had been produced at heights of seventeen, twenty-seven, and thirty-four inches from the root collar. The decayed wood was a cream color in contrast to the reddish brown of the normal wood. When freshly cut the decayed area was surrounded by a faint violet-colored band which disappeared upon drying.[1] Infection appeared to have taken place

[1] In one other instance this colored band was noted, namely, in the cross section of a cherry tree in which the fungus was causing a heart-rot. Here the entire decayed area was surrounded by the violet band.

just above the level of the ground where the exposed sapwood was more moist than higher up in the scar. No decay was found in the root of the tree. Only a small amount of extreme decay was present in the trunk. The incipient stage occupied the greater area, that is, the wood rays were quite firm throughout, although the elements between them crumbled readily.

The material to be sectioned was treated in several ways. Some of the wood was put in chromo-acetic acid, washed, and subjected to the action of hydrofluoric acid for five weeks, then washed and embedded in celloidin. Other material was treated with chromo-acetic acid and preserved in 70% alcohol. Some of the wood was used with no special treatment of any kind. The sections from acid treated wood reacted to the stains similarly to the untreated material, except that the stains were less brilliant. Wood was macerated according to Schultze's method (11).

Microscopic Studies.— The wood of white ash is brownish with a lighter brown sapwood, ring porous, close grained, hard, and tough. Radiating outward are two kinds of wood rays, the uniseriate and biseriate, which are several to many cells high. The spring wood contains large vessels surrounded by wood parenchyma and tracheids. In the summer wood are small thick walled vessels arranged in pairs and surrounded by paratracheal parenchyma, which extends tangentially from one group of vessels to another. Wood parenchyma also terminates each annual ring. The remainder of the xylem consists wholly of fibers.

Decayed wood of white ash is readily distinguished from sound wood because of its bleached appearance. In general there is an abrupt change from normal to decayed wood. The early and late stages of decay are similar in color but, as decay advances, the strength of the wood decreases, and it is less resistant to cutting and breaking. The rays in the early stage of decay are firm and brownish in color; in extreme decay they are bleached and readily crumbled. Viewed under the microscope the first evidence of decay is the thinning of the fiber walls. As decay advances the secondary walls of the fibers are gradually dissolved until only the middle lamellae remain. In some of the cells the walls are digested evenly, in others they are dissolved in an irregular manner. In a given area, decay takes place at about the same rate in the fibers attacked. In extreme decay, however, it is possible to find some fibers which show no evidence of disintegration even though all the surrounding cells have been practically destroyed. The tracheids are the next elements attacked, and the digestion of their walls progresses as it does in the fibers, although considerably slower. Possibly the tracheids are more highly lignified as certain stains seem to indicate. There is no noticeable decrease in the thickness of the walls of the vessels, wood parenchyma, or ray cells even in extreme decay. The walls of these elements are badly perforated by fungi hyphae which in places are so numerous that they join and give the appearance of ragged tears. The perforations are larger than the diameter of the

hyphae and, as Dr. Kauffman (24) suggests, this may be due to the fact that the enzymes continue to act upon the walls after the hyphae have passed through or even disappeared. The lumina of numerous vessels, in both early and late stages of decay, are filled with compact masses of hyphae. These hyphae are of two distinct sizes, large hyphae which do not branch profusely and smaller hyphae from which numerous branches develop. The latter are very often present in the other elements.

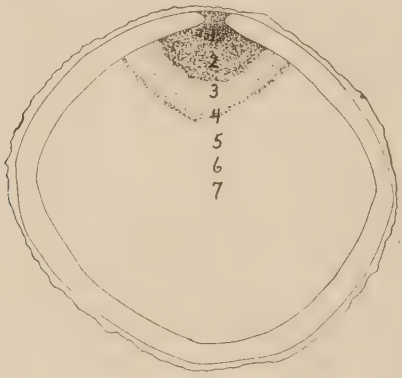

Fig. 1.

Cross section from trunk of white ash infected with *Polyporus gilvus*. The numbers indicate the positions from which the blocks were removed for study. Stippling represents the area of decay.

As indicated in Figure 1, seven blocks of wood were chosen for study, ranging from extreme decay to apparently sound wood. A block of wood from a higher level in the trunk where there was no decay present, was used as a check as well as wood from other sources. After testing in every way with stains it was found that wood from block seven reacted similarly to normal ash wood, hence this block was used to represent normal wood in the following studies.

Presence of Hyphae in Decayed Wood.—As soon as the blocks were removed from the trunk, tissue cultures were attempted from each. Where there was mycelial growth the hyphae were carefully examined, transfers were made to other culture tubes and the growth of the mycelium watched and compared with the cultures from single spores. There was no question in the author's mind but that, where cultures were obtained, they were of *Polyporus gilvus,* although none were carried through to the fruiting stage.

TABLE 4.—*The blocks are those indicated in Figure 1.*

Blocks	1	2	3	4	5	6	7
Hyphae	Present	Present	Present	Present	Absent	Absent	Absent
Cultures	Positive	Positive	Positive	Positive	Negative	Negative	Negative

Hyphae were present in the wood which appeared bleached, extending from early stages of decay to extreme decay as shown by microscopic studies and positive cultures of the fungus. The following stains proved useful in locating the hyphae in the wood: Haidenhain's haematoxylin-safranin (24), safranin and light green (35), magdala red and light green (13), malachite green and acid fuchsin (6), bismark brown and methel violet (22), and eosin. Positive cultures showed that these hyphae were still alive even in extreme decay. Studies of certain other rots have indicated that hyphae of the invading fungi disappear from extermely decayed areas of the host woods. Block number four was taken from the region which was violet in color when freshly cut, but which, upon drying, took the typical color of sound wood. To the unaided eye this appeared to be normal wood, and under the microscope showed no thinning of the fiber walls, even though hyphae were present. This zone marked the advance of the hyphae toward the sound wood. If a section of the trunk was kept moist this violet colored zone was marked by the early appearance of aerial hyphae, indicating that the more actively growing hyphae were apparently concentrated in this region.

Within the cells, the hyphae tended to grow lengthwise of the cavities except in the large vessels where they branched in all directions. Within the vessels the hyphae appeared larger than in the other elements. The larger hyphae were from hyaline to light brown in color, the smaller hyphae were all hyaline. In passing from cell to cell they were not limited to the pits but passed through the walls at any point.

Microchemical Stains.— The usual chemical stains were used for this work, only two of which will be recorded here, one because it checks so closely with the chemical analysis and the other because the results are to the contrary, which intimates that microchemical stains are poor indicators of chemical changes in wood.

TABLE 5.— *Result of staining for cellulose and lignin in normal wood of white ash with Haidenhain's haematoxylin-safranin.*

Elements	*Cellulose*	*Lignin*
Fibers	Primary walls deep blue	Secondary walls red to violet red.
Tracheids	Primary walls blue; secondary walls tinged with faint blue.	Secondary walls red to violet red.
Vessels	Primary walls blue	Secondary walls red.
Wood parenchyma	Primary walls blue; end walls blue.	Secondary walls red tinged with faint blue.
Ray cells	Primary walls blue; occasional cells were faint blue.	Most cells stained deep red.

TABLE 6.— *Result of staining for cellulose and lignin in decayed wood of white ash with Haidenhain's haematoxylin-safranin.*

Elements	*Cellulose*	*Lignin*
Fibers	Primary walls blue; remaining fragments of secondary walls blue.	Remaining fragments of secondary walls give no lignin test.
Tracheids	Primary walls blue; secondary walls thinner and irregularly blue from within toward lamella.	Secondary walls irregularly pink from lamella to lumen.
Vessels	Primary walls blue	Secondary walls red.
Wood parenchyma	Primary walls blue; end walls blue.	Secondary walls red tinged with faint blue.
Ray cells	Primary walls blue; occasional cells a faint blue.	Most cells a deep red.

In normal wood the middle lamellae of all the cells stained a deep blue with haematoxylin. The wood parenchyma gave a faint violet color, which was also slightly perceptible in the ray parenchyma and tracheids. The secondary wall of the tracheids is so thin that possibly the blue tinge came from the heavily stained lamella.

In decayed wood the blue is more noticeable over the entire section. The lamellae remained a deep blue. There was no noticeable change in the color of the wood parenchyma. The inner walls of the fibers had almost wholly disappeared, leaving the blue-black primary walls and fragments of the secondary walls which were a distinct blue. The walls of the tracheids were partially digested leaving blue fragments of the secondary walls. The ray parenchyma appeared much the same as in the sound wood, or slightly more violet if there was any change.

In using Haidenhain's haematoxylin-safranin, certain investigators state that the blue indicates the presence of cellulose, and that the lignified tissue stains red. Interpreting accordingly, the first action of the fungi enzymes is to delignify the fiber and tracheid walls and then gradually to digest them, leaving, in extreme decay, only a cellulose skeleton of the wood, with non-delignified vessels, rays, and wood parenchyma. This result might well be accepted but for the fact that the middle lamella stains blue to blue-black in both normal and decayed wood, indicating that it is cellulose in nature, and it has recently been shown that the middle lamella is highly lignified (37). In view of this fact and the color changes noted, it would hardly be wise to draw definite conclusions from the above stain reactions, since here blue did not necessarily indicate cellulose.[1]

[1] Sections of decayed and normal wood were passed through the stains, dehydrating and clearing agents, in groups. It had been previously experienced that if consecutive sections from sound wood were left in haematoxylin for slightly varying periods of time, the intensity of the blue varied accordingly. On the other hand, if the sections remained in alcohols for small differences of time, the safranin stain would vary from pink to deep red. In order, then, to draw any conclusions through comparison concerning the chemical changes occurring during decay, it was essential for the sections to have exactly the same periods of time, and this could only be accomplished by running the sections through the stains in groups.

TABLE 7.— *Result of staining for cellulose and lignin in normal wood of white ash with phloroglucin-HCl and chlorzinc-iodine.*

Elements	*Cellulose*	*Lignin*
Fibers	Secondary walls faint blue to colorless.	Primary walls deep red; secondary walls pink to mostly colorless.
Tracheids	Secondary walls faint blue to colorless.	Primary walls deep red; secondary walls change from deep red at lamella to pink or colorless at lumen.
Vessels	No cellulose test	Primary and secondary walls a deep red.
Wood parenchyma	Light blue end walls	Primary and secondary walls deep red. End walls faint pink to mostly colorless.
Ray cells	Some cells a violet red	Rays mostly a deep red.

TABLE 8.— *Result of staining for cellulose and lignin in decayed wood of white ash with phloroglucin-HCl and chlorzinc-iodine.*

Elements	*Cellulose*	*Lignin*
Fibers	Remaining fragments of secondary walls a deep blue.	Remaining fragments of secondary walls faint pink to colorless. Primary walls red.
Tracheids	Secondary walls thinner and blue to faint blue.	Primary walls red. Secondary walls merge from red near lamella to pink or colorless at lumen.
Vessels	No cellulose test	Primary walls and secondary walls deep red.
Wood parenchyma	Light blue end walls	Primary and secondary walls deep red.
Ray cells	Some cells a violet red	Rays mostly a deep red.

Phloroglucin-HCl and chlorzinc-iodine were used individually and not as conbination stains, although the results are recorded together in the above tables.

Phloroglucin-HCl.— In normal wood the middle lamellae of all the cells stained a deep red. The secondary walls of the vessels, wood parenchyma, and ray cells stained red. The secondary walls of the fibers became a faint pink or remained colorless, those of the tracheids, a light pink.

In decayed wood the middle lamellae remained a deep red in all of the cells. The secondary walls of the vessels, wood parenchyma and ray cells remained red. The secondary walls of the fibers and tracheids had been partially digested, the fragments which did remain were a faint pink to colorless. The only apparent change being, then, in the digestion of the secondary walls of the tracheids and fibers, the fiber walls being digested before the tracheids were attacked.

Chlorzinc-iodine.— In normal wood the middle lamellae, entire walls of the vessels, wood parenchyma, and ray cells stained from yellow to golden yellow. The secondary walls of the fibers became a light blue and those of the tracheids a faint blue. The end walls of the wood parenchyma cells stained blue. Occasional ray cells showed a blue color.

In decayed wood the only apparent change from that of normal wood was in the fibers and tracheids. The secondary walls of both were mostly digested, the remaining fragments being colored blue.

The faint pink in the fiber and tracheid walls, when treated with phloroglucin-HCl, and the pale blue reaction in response to chlorzinc-iodine, indicated that they were only slightly lignified. Many of the fiber walls showed no pink at all, but stained a distinct blue, suggesting the absence of lignin in those walls. The chemical changes indicated by these stain reactions was the delignification of the lightly lignified fiber and tracheid walls, followed by the digestion of these parts, leaving only a skeleton of highly lignified tissue in extremely decayed wood.

As previously stated, various other microchemical stains were employed, most of which entirely agree with the changes indicated by the use of chlorzinc-iodine and phloroglucin-HCl. However, the author did not feel justified in drawing definite conclusions concerning the chemical changes from the stain reactions alone, especially when faced by the fact that even phloroglucin–HCl and chlorzinc-iodine are not infallible as indicators of the chemical changes in wood which take place during the process of decay (40).

Sulphuric Acid Tests.— Sections corresponding to those used with the microchemical stains were placed upon slides and treated with 72% sulphuric acid as described by Ritter (37). The treatment accorded all sections was the same.

Sections taken from blocks one, two and three represented decayed wood. The fragments of the secondary walls of the fibers and tracheids in these sections dissolved and disappeared from the field, leaving a network of middle lamellae, vessels, rays, and wood parenchyma cells in place on the slide. Sections taken from blocks, five, six, and seven, represented normal wood. Immediately after these sections were treated with acid, the cells swelled rapidly, broke apart, and the secondary walls of the fibers and tracheids dissolved, leaving on the slide a mixture of floating lamellae, vessels, rays, and wood parenchyma cells. The violent action in the latter case was due to the presence of the large amount of cellulose in the cell walls of the sound wood. The swelling of this cellulose preceding dissolution forced all the elements apart. In the decayed wood the action was so calm that the elements remained in the normal position. The conclusion was that there is less cellulose in the decayed wood than in sound wood, hence the less violent chemical action. This test was not conclusive in itself, but at least indicated the presence of less cellulose in wood decayed by *Polyporus gilvus* than in normal wood.

Chemical Analysis.— The ash log, from which the small blocks had been removed for the previous studies, had been kept in the laboratory, and the wood from this was used for the chemical analysis. That is, the wood used for the chemical analysis and for the microchemical stain reactions, came from the same log and as near the same position in that log as it was possible to obtain

it. This work was done by Mr. W. Willets in the Department of Forest Chemistry of the New York State College of Forestry under the supervision of Dr. L. E. Wise. The samples of the normal wood and the decayed wood were prepared by the author and given to Mr. Willets, with no hint as to what the stain reactions had indicated. Following is his report as given to the author.

Methods of Analysis.— Sawdust from decayed wood and normal wood was used as samples. It was passed through a grinder and sieved. The material, passing a forty mesh sieve, but not a sixty mesh sieve, was chosen for the purpose of the analysis.

(1) Moisture. Moisture was determined by the method advocated by Dore (50, 1919, 1920[2], [3]; 49), a two-gram sample being dried at 100° C. for sixteen hours to constant weight in a glass-stoppered weighing bottle, which was sealed and then cooled in a dessicator. It is important that all samples of dried sawdust, cellulose, etc., be sealed and weighed in a weighing bottle, since they are rather more hygroscopic than the dessicating agent in the dessicator.

2. Ash. The ash was determined by the incineration of a one gram sample in a porcelain crucible in an electric furnace, the general procedure in determining the ash of any organic material.

(3) Benzene Alcohol Extract. A one and a half (1.5) gram sample was weighed into an alundum thimble and extracted for six hours in a Soxhlet extractor with a mixture of equal volumes of redistilled benzol and alcohol (53). The extract was then transferred to a tared Erlenmeyer flask (100 cc.) and the solvent evaporated on a water bath. As soon as the solvent had evaporated the flask was removed to an electric oven, and further dried for exactly one hour at 100° C. and weighed.

(4) Lignin. A one and a half gram extracted sample was weighed into an 800 cc. beaker, and 20 cc. of sulfuric acid (72%) added. The hydrolysis was allowed to proceed at room temperature for three and one-half hours; 50 cc. of cold distilled water, followed by 500 cc. of hot distilled water were added. The residue was filtered on a tared Gooch crucible with an asbestos mat, and thoroughly washed with hot water to remove all traces of the acid. The sample was dried in the oven at 100° C. for sixteen hours and weighed (50, 1920[3]). This method was also used to determine the residual lignin in the cellulose after chlorination.

(5) Cellulose. In general, Schorger's (53[1]) method was followed in the determination of cellulose, however, there were certain modifications employed, so the process will be described.

A one gram sample of extracted material was weighed into a beaker (250 cc.) and slightly moistened with distilled water. Chlorine gas was passed into the beaker for three minutes and the sample stirred constantly, 5 cc. of sodium sulfite solution (3%) was added to the beaker and the sample placed on a water bath for forty-five minutes. The sample was then transferred to a tared Gooch crucible, and the sodium sulfite solution poured through, followed by a washing with hot water on suction. The

sample was then transferred to the original beaker, and the process was repeated, all subsequent filterings and washings being in the original crucible. The average number of chlorinations necessary to remove the lignin was four, the maximum number being five. The crucible was then dried in a weighing bottle in the oven for sixteen hours at 100° C., sealed, cooled in a dessicator and weighed.

Residual lignin was determined according to the method given on page 29 of this report. In every case the percentage was below one per cent; in all but two cases the quantity was so small that it could not be weighed.

Alpha (normal) cellulose was determined by the addition (to the Cross and Bevan cellulose) of sodium hydroxide solution (17.5%) in the ratio of 5 cc. of the alkali to one gram of the sample. The alkali and cellulose were thoroughly incorporated and allowed to stand at room temperature (which was observed to be 19° C. in each case) for one-half hour. The sample was then transferred to a tared Gooch crucible, washed with sufficient cold water to remove traces of the alkali, then with acetic acid, and finally with sufficient cold water to remove all traces of the acid. It was then dried at 100° C. and weighed as alpha (normal) cellulose.

(6) Hot water soluble (53[1]; 52). A one gram sample was weighed into an Erlenmeyer flask, and digested with water (at the boiling point) for three hours under a water-cooled reflux condenser. The sample was then transferred to a tared Gooch crucible, washed with hot water, dried and weighed, the loss in weight being the hot water soluble material present in the original sample.

(7) Cold water soluble (53[1]; 52). A one gram sample was digested with 300 cc. of cold water at room temperature for twenty-four hours. It was then transferred to a tared Gooch crucible, washed with cold water, dried at 100° C. and weighed.

(8) Alkali soluble (50, 1920[3]). A one gram sample was treated with sodium hydroxide solution 5% (100 cc.) for twenty-four hours at room temperature. The sample was transferred to a tared Gooch crucible, washed with cold water, acetic acid (5%) and again with sufficient cold water to remove all traces of the acid. It was then dried at 100° C. and weighed, the loss in weight being the alkali soluble material present in the original sample.

(9) Pentosans. The regular Tollen's Method for determining pentosans was employed (53[1]). The weight of pentosans obtained was calculated from the tables of Kröber (Browne's Handbook of Sugar Analysis, Appendix, 83–88) and also from Kröber's formula (Browne's Handbook of Sugar Analysis, 452).

Results.— The following tables give the results of the analysis. They are calculated for both air dry and oven dry (100° C.) samples.

Table 13 gives the oven dry analysis of the decayed wood recalculated on the basis of the sound wood, i. e., as if the lignin

remained constant. This was a necessary assumption since it is certain that no lignin was synthesized during the decay of the wood. The method for calculating Table 13 was the same as that employed by Dore (51), although in this case it was not necessary to recalculate to an ash-free, nitrogen-free basis.

TABLE 9.— *The analysis of normal wood, air dry-basis.*

	I	*II*	*III*	*IV*	*Av.*
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Moisture	4.66	4.68	.....	.....	4.67
Ash	0 545	0 534	.....	.....	0.539
Benzene-alcohol soluble	4.21	4.23	4.26	4.26	4.24
Lignin	20.28	19.99	19.87	19.97	20.03
Cellulose (corrected for lignin)	48.99	49.33	48.41	48.34	48.76
Alpha cellulose	36.74	37.16	36.55	36.28	36.68
Hot water soluble	3.88	3.77	.....	.....	3.83
Cold water soluble	1.91	1.84	.....	.....	1.88
Alkali soluble	20 36	20.38	.....	.....	20.37
Pentosans	20.20	20.03	.....	.....	20.12

TABLE 10.— *The analysis of normal wood, oven-dry basis (100° C.).*

	I	*II*	*III*	*IV*	*Av.*
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Moisture	4 87	4.91	.....	.....	4.88
Ash	0.573	0.561	.....	.....	0.567
Benzene-alcohol soluble	4.43	4.45	4.48	4.48	4.46
Lignin	21 19	21.03	20.95	21.00	21.03
Cellulose (corrected for lignin)	51.41	51.76	50.95	50.83	51.24
Alpha cellulose	38.55	39.19	38.41	38.57	38.68
Hot water soluble	4.08	4.00	.....	.....	4.04
Cold water soluble	2 01	1.99	.....	.....	2.00
Alkali soluble	21.29	21 32	.....	.....	21.30
Pentosans	21.13	20.96	.....	.....	21.05

TABLE 11.— *The analysis of decayed wood, air-dry basis.*

	I	*II*	*III*	*IV*	*Av.*
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Moisture	5.54	5.54	.....	.....	5.54
Ash	0 547	0.528	.....	.....	0.538
Benzene-alcohol soluble	2.55	2.49	2.50	2.44	2.50
Lignin	25.10	24 80	25.04	25.13	25.02
Cellulose (corrected for lignin)	43.89	43.62	43.41	43.29	43.55
Alpha cellulose	34.98	35.12	34.78	34.63	34.88
Hot water soluble	2 85	2 80	.....	.....	2.83
Cold water soluble	1.38	1.42	.....	.....	1.40
Alkali soluble	23.10	23.14	.....	.....	23.12
Pentosans	23 31	23.49	.....	.....	23.40

TABLE 12.— *The analysis of decayed wood, oven-dry basis* (100° C.).

	I	*II*	*III*	*IV*	*Av.*
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Moisture	5.87	5.87	.....	.....	5.87
Ash	0.582	0.561	.....	.....	0.572
Benzene-alcohol soluble	2.70	2.66	2.65	2.55	2.64
Lignin	26.67	26.55	26.76	26.69	26.67
Cellulose (corrected for lignin)	46.61	46.28	46.01	45.99	46.23
Alpha cellulose	37.04	37.26	36.86	36.83	36 99
Hot water soluble	2.96	2.90	.....	.....	2.93
Cold water soluble	1.46	1.51	.....	.....	1.49
Alkali soluble	23.74	23.79	.....	.....	23.77
Pentosans	23.02	23.23	.....	.....	23.13

TABLE 13.— *The analysis of decayed wood recalculated on the basis of the normal wood. (Oven-dry, constant lignin).*

	I	*II*	*III*	*IV*	*Av.*
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Moisture	4.60	4.65	.....	.....	4.63
Ash	0.456	0.443	.....	.....	0.450
Benzene-alcohol soluble	2.12	2.11	2.08	2.01	2.08
Lignin	21.03	21.03	21.03	21.03	21.03
Cellulose	36.54	36.65	36.16	36.24	36.40
Alpha cellulose	29.03	29.51	28.97	29.02	29.13
Hot water soluble	2.31	2.29	.....	.....	2.30
Cold water soluble	1.15	1.19	.....	.....	1.17
Alkali soluble	18.75	18.79	.....	.....	18.77
Pentosans	18.19	18.35	.....	.....	18.27

TABLE 14.— *The average analysis, oven-dry basis.*

	Normal	*Decayed*	*Decayed (Recalculated)*	**Decrease*
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
Moisture	4.88	5.87	4.63	5.1
Ash	0.567	0.572	0.450	20.06
Benzene-alcohol soluble	4.46	2.64	2.08	53.3
Lignin	21.03	26.67	21.03	0.0
Cellulose	51.24	46.23	36.40	29.8
Alpha cellulose	38.69	36.99	29.13	24.6
Hot water soluble	4.04	2.93	2.30	43.06
Cold water soluble	2.00	1.49	1.17	41.5
Alkali soluble	21.30	23.77	18.77	11.9
Pentosans	21.05	23.13	18.27	13.2

Discussion of Results.— In the discussion of results, only the analysis of the sound wood, and the recalculated analysis of the decayed wood will be considered. To secure data for the purposes of comparison, it is important to bear in mind that lignin cannot be synthesized in the process of decay, and therefore the original analysis of the decayed wood should be recalculated on the basis of the sound wood, i. e., on a constant lignin basis.

The benzene-alcohol soluable material decreased during the process of decay, approximately half the amount present in the normal wood being in evidence in the decayed wood. It is therefore probable that the fungus made use of these materials in its life processes.

This was also the case with the hot and cold water soluble material, which decreased almost in the same proportion, this proportion, however, less than in the case of the benzene-alcohol soluble material. It is well to note that the chemical nature of these extractives (or extraneous materials) in the wood of *Fraxinus alba* is as yet unknown.

The alkali soluble material is supposed to consist mainly of the pentosans and other furfural-yielding materials. This would seem to be the case from the analyses, since the percentage of pentosans and the alkali soluble material was approximately the same. It

* Calulated on the basis of the recalculated analysis of the decayed wood, and on the analysis of the normal wood.

is important to note that this is a more or less speculative hypothesis, and has no definite proof. The pentosans (as well as the alkali soluble material) decreased during the process of decay, however, the percentage decrease was not as great as in the case of the other soluble (i. e., extraneous) materials.

The cellulose content also decreased in a lesser proportion than the benzene-alcohol and water-soluble constituents but in a greater proportion than the pentosans and alkali soluble components. The percentage of decrease in the case of the alpha cellulose was slightly less than in the case of the Cross and Bevan cellulose, this might reasonably be assumed because of the greater resistance of the alpha cellulose.

It seems evident, therefore, that the fungus derived its major portion of food from the carbohydrate material present in the wood (in the cell wall, as in the case of cellulose and the pentosans; and the extraneous materials), leaving the legnin apparently unattacked.

The results of the analysis of the sound wood of *Fraxinus alba* as herein given, concur reasonably well with the analysis as published by the Forest Products Laboratory at Madison, Wisconsin.

Summary.— The chemical changes in the wood of *Fraxinus alba* during its infection by *Polyporus gilvus* (Schw.) Fries may briefly be summarized as follows:

(1) The lignin component of the wood appeared to remain unattacked during decay, judging from its apparent accumulation.

(2) The extraneous materials of the wood decreased in varying proportions.

(3) The cellulose component of the wood decreased.

(4) The alpha cellulose component of the wood decreased.

(5) The main source of food of the fungus was the carbohydrate material in the wood.

The entire quantity of sawdust prepared for the chemical analysis was not used for that purpose. Of that which remained, equal volumes of the decayed and normal sawdust were weighed to determine the "volatile loss" as defined by M. W. Bray (7). Using the formula he suggested,

$$\frac{\text{"Percentage of lignin in sound wood} \times 100}{100\text{—"Volatile loss"}} =$$

caluculated percentage of lignin in sample as analyzed," it was found that the result obtained checked very closely with that of the chemical analysis (24.31% lignin calculated figure as against 25.02% actual analysis, air dry basis). The "volatile loss" was 17.60%, hence 1 gm. of sound wood weighed 8.240 mgm. when decayed. One gram of normal wood contained 200.3 mgm. of lignin (Table 9) but when decayed it weighed 8.240 mgm. with 24.31% lignin, or 200.3 mgm., therefore the actual weight of lignin in decayed wood and sound wood was the same. Evidently the lignin lost during the digestion of the lightly lignified secondary walls of the fibers and tracheids, and the perforating of the highly lignified walls, is so small that it is not measurable.

The chemical analysis bears out what the majority of the microchemical stains indicated, namely that the main action of the fungus during the process of decay is the destruction of the cellulose, leaving the strongly lignified elements practically unaffected.

Decay of White Oak

Macroscopic Studies.— White oak is commonly attacked by *Polyporus gilvus*. Sporophores of the fungus are found in abundance upon wood of both dead and living trees. A living oak tree bearing sporophores of the fungus was selected for study. The tree selected was chosen for two reasons, first because the decay was representative of oak wood attacked by this fungus, and secondly, because it gave an opportunity to make a study of parasitism by the fungus.

The oak was forty feet high, ten inches in diameter at the base and showed eighty annual rings in cross section. Fifty-seven sporophores were scattered over the bark on the west side of the tree, the majority of which were stratified, showing two layers of tubes. Ten of these were partially over-grown by young mycelium so that at the end of the summer they would have shown three distinct layers of tubes. There was no evidence of any sporophores having been broken off hence it was concluded that this was probably the third season that fruiting bodies had been produced. Decay had undoubtedly been going on prior to the production of sporophores. An old lightning scar was found on the west side of the trunk, extending from the top to the root collar. The roots on this side of the tree were dead and the sapwood decayed. Just above the root collar some of the bark had dropped off from the lightning scar, exposing the dead sapwood. Above this point the bark was intact but could be easily removed in the area of the scar. Living branches were found only on the east, north, and south sides of the trunk. Those on the west side were dead and badly decayed.

The tree was felled and cut into short lengths for study. Just above the root collar and within the lightning scar, decay extended through the sapwood into the heartwood. In the sapwood, it extended to the right and left within the limits of the scar. Extreme and incipient decay were present. No black lines of decay nor any colored lines indicating the boundary between decayed and sound wood were detected. Decay extended through the trunk to the topmost branch, but decreased in depth and width as it reached upward. Evidence pointed toward the fact that infection had taken place just above the root collar where the exposed sapwood was sufficiently moist to support germination of the spores.

Fifteen feet above the ground a number of sporophores were grouped together. They were producing spores as white deposits beneath them indicated, showing that the mycelium was alive within the wood at this level. A cross section of the trunk was made at this place and it showed all stages of decay present as

well as apparently sound wood. Blocks were removed as indicated in Figure 2. These blocks were split in two longitudinally, and half the group were treated similarly to the blocks of white ash, the remaining half was used for a study of the fresh wood.

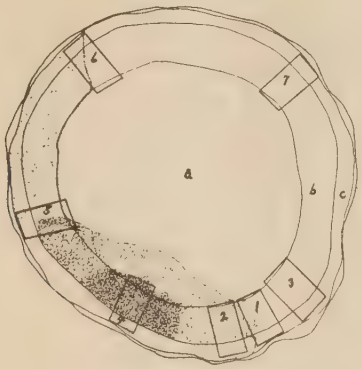

Fig. 2.
Cross section of white oak trunk to indicate areas from which blocks were removed for study. Stippling represents decay. (a) Heartwood. (b) Sapwood. (c) Inner bark.

Microscopic Studies.— The wood of white oak is strong and durable. The brown heartwood is readily distinguished from the lighter sapwood which occupies about twenty per cent of the wood volume. Large thick-walled vessels, filled with tyloses, are found in the spring wood, and these give way abruptly in the summer wood to the small, thin-walled, angular vessels which are arranged in groups. Tracheids, fiber tracheids and libriform fibers make up the ground mass of the wood through which the wood parenchyma is diffused. The tracheids are most numerous within the spring wood about the larger vessels. Two types of rays are present, uniseriate and biseriate.

Decayed wood showed the presence of hyphae in the various elements, especially abundant within the larger vessels. The first evidence of decay in the woody tissue was the gradual thinning of the fibers, the secondary walls of which were digested outward from the lumen. Most of the fibers in any one area were in approximately the same stage of disintegration. The tracheids were the next elements attacked but were much more resistant to decay than the fibers. The wood parenchyma cells scattered among the fibers and tracheids showed no evidence of decay other than the numerous perforations in the walls through which the hyphae passed. The vessels and ray cells persisted in extreme

decay without any apparent thinning of the walls, even though they were full of perforations. The final stage of decay presented the appearance of a network of middle lamellae, with vessels, rays and wood parenchyma cells still present. With forceps it is possible to remove sections of large vessels several centimeters long. The large rays broke apart easily and single cells could be teased out of the mass for examination. The entire wood presented a bleached appearance and turned a brilliant red when treated with phloroglucin–HCl.

It is worthy of note that single-spore cultures upon blocks of white oak have been carried on for several years. Fruiting bodies were produced upon the wood and the fungus finally died, presumably from starvation. The wood of these blocks was bleached, brittle, and fully represented extreme decay as found in nature. The wood when tested with various stains gave no evidence of the presence of cellulose but responded strongly to the lignin tests. These results point to the fact that the decay associated with *Polyporus gilvus* in nature is actually caused by that fungus.

Presence of Hyphae in Decayed Wood.— In the oak wood as in ash, hyphae were present in extreme decay. Sections from blocks indicated in Figure 2, showed hyphae to be present in blocks one, two, four, five and six. The hyphae were more abundant in extreme decay than in the area of incipient decay. That this mycelium was that of *Polyporus gilvus* and, that it was still alive, was proven by tissue cultures obtained from the above mentioned blocks at the time they were cut. The hyphae were most abundant in the vessels, wood parenchyma, and ray cells. These were the elements which appeared to be the least affected, another circumstance indicating that the fungus does not seek out the lignin as food material, for if it did, surely these highly lignified elements would be the first to be digested during the process of decay.

Hyphae were abundant throughout the dead bark covering the decayed sapwood. In the bark they were massed in groups so that to the unaided eye they appeared as brown threads. Masses of hyphae occupied the region of the phloem parenchyma and often enclosed groups of bast fibers and stone cells which remained apparently undigested. In the region of the bark it should be stated that the fibers and stone cells were very highly lignified as shown by certain microchemical stains. That some change had taken place in the fibers was evident, however. In the bark where hyphae were present, the masses of fibers stood out as white patches in contrast to the brown color of the other elements; that is, the same bleaching effect had taken place in the woody tissue of the bark as had occurred in the xylem proper.

Microchemical Stains.—The results of only two microchemical stains are recorded here, the two which agreed with the results of the chemical analysis of the ash wood.

TABLE 15. — *Result of staining for cellulose and lignin in normal wood of white oak with phloroglucin-HCl and chlorzinc-iodine.*

Elements	*Cellulose*	*Lignin*
Vessels	Tyloses faint blue to colorless at times.	Red to colorless tyloses. Primary and secondary walls deep red.
Fibers	Secondary walls faint blue to colorless.	Primary walls red. Secondary walls faint pink to colorless.
Tracheids	Some secondary walls faint blue.	Primary walls red. Secondary walls red near lamella, pink or colorless at lumen.
Wood parenchyma	Blue to colorless end walls	Primary walls red. Secondary walls a lighter red. Pink to colorless end walls.
Ray cells	Some rays give faint blue to lavender.	Most rays stain red.

TABLE 16.— *Result of staining for cellulose and lignin in decayed wood of white oak with phloroglucin-HCl and chlorzinc-iodine.*

Elements	*Cellulose*	*Lignin*
Vessels	Tyloses faint blue to colorless.	Pink to colorless tyloses. Primary and secondary walls deep red.
Fibers	Remaining fragments of secondary walls blue to colorless.	Primary walls red. Fragments of secondary walls faint pink to mostly colorless.
Tracheids	Thinner secondary walls faint blue.	Primary walls red. Secondary walls pink to colorless.
Wood parenchyma	Blue to colorless end walls. Some secondary walls faint blue.	Primary walls red. Pink to colorless end walls. Secondary walls red to pink.
Ray cells	Some rays give faint blue to lavender.	Most rays stain red.

As Table 16 shows, the process of decay in white oak is similar to that in ash wood. The cellulose walls and lightly lignified walls are the parts digested by the fungal enzymes. The heavily lignified parts remain.

Tyloses have generally been considered as cellulose in nature. In the oak wood used for this work, the tyloses were commonly lignified. This was to be expected in view of the fact that the tyloses consist presumably of the primary wall only, that is, of the middle lamella. Some sections gave evidence of the fact that there was more thickness to the walls of the tyloses than the lamella itself afforded, and certainly some of them gave a faint blue color when treated with chlorzinc-iodine. It is merely suggested that possibly there was a thin secondary wall consisting of cellulose material, and that this responded to the test for cellulose. Tyloses remained in extremely decayed wood but were ordinarily so filled with perforations that they fell apart when the celloidin was removed from the sections.

It was previously stated that decayed wood splits along the annual rings. This can now be explained by the fact that sections from extreme decay show the summer wood to be a mere network of middle lamellae, and it is in this portion of the annual ring that the decayed wood breaks.

Sulphuric Acid Tests.— Sections taken from the blocks were treated with 72% sulphuric acid as previously described. Again the decayed wood reacted very slowly to the treatment, whereas the normal wood broke up as it did in white ash, due to the violent chemical action upon the cellulose, which was apparently present in greater quantity in the sound wood.

No chemical analysis of oak wood was made. The results of the microchemical stains upon white oak and white ash agreed in every respect; the response to the treatment with sulphuric acid was the same; the elements remaining in extremely decayed wood were of the same type; hence it is logical to expect that a chemical analysis would show a greater proportion of lignin and a smaller amount of cellulose in decayed wood than in sound wood of white oak.

Discussion

The chemical analysis of the decayed wood showed a decrease in the amount of cellulose as compared with the amount in sound wood. Other white rots have given similar results (20) showing clearly that there are exceptions to the widespread opinion that white rots indicate delignification and brown rots the loss of cellulose. The above results might have been anticipated since the secondary walls of the fibers and tracheids were the elements destroyed, leaving the middle lamellae and other highly lignified parts. The most extreme decay would undoubtedly have shown a greater proportion of lignin present with the accompanying loss of cellulose. The wood used for the chemical analysis, while severely decayed, was not decayed beyond the point where, with extreme care, it could be sectioned sufficiently well to be tested with microchemical stains. This stage of decay was chosen since it was felt that, for comparison, it was essential to have wood that could be used advantageously in both kinds of tests. Wood in the last stages of decay could not be sectioned unless it was embedded in celloidin; however, a few crude sections were obtained from the untreated wood and microchemical tests were performed upon them. When treated with the various reagents, the elements responded strongly to the lignin tests, but seldom reacted to the tests for cellulose.

It will be noted that in the woods used, the secondary walls of the fibers were apparently not lignified, or if so, only very lightly. This fact is borne out by the microchemical stains as well as the results of the chemical analysis. Spaulding (42, 1906) found this to occur only exceptionally in the woods he studied. In the many specimens of white oak and white ash used in this work, it was the exception to find these elements lignified to the extent of reacting strongly to the test with phloroglucin–HCl. In the same paper Spaulding also states, "If there is any cellulose layer present in the wood before it is attacked it is probably the first part of the wall to be dissolved, and it is fairly certain that it does not exist in the last stages of decay." He illustrates this point by wood from the tree of *Carpinus caroliniana* attacked by *Polu-*

porus gilvus. He states, "At the edge of the affected wood was a narrow border of dark, infiltrated cells separating the healthy from the diseased wood. In the healthy wood just outside this infiltrated zone the wood fibers in many cases had an inner, lining layer of cellulose. This layer disappeared in the infiltrated zone while in the very badly rotted tissue *the cell walls consisted only of cellulose, the lignin appearing in greater and greater quantity as the healthy wood was approached.*" He does not state whether the secondary walls were simply delignified or whether they were partially digested as the "inner lining" or the tertiary walls. If the inner cellulose layer is rapidly digested then it is logical to expect the secondary layer to be digested as well if it consists of "only pure cellulose" after delignification. If this was not the case then it would seem that this was an incipient stage of decay and not "badly decayed wood." In the ash and white oak wood used for this study, the secondary walls of the fibers were the first elements attacked, and they were rapidly digested. Badly decayed wood showed only fragments of this wall. The primary walls remained intact and were highly lignified, not "pure cellulose" as Spaulding states for decayed wood of *Carpinus*. Decay of red oak[1] was similar in that the tertiary walls of the gelatinous fibers were the first to be dissolved and the secondary walls next, again leaving a skeleton of highly lignified elements in the last stage of decay. Unquestionably *Polyporus gilvus* is capable of delignifying cell walls when they are only lightly lignified, but probably this is only incidental to the digestion of the cellulose. Where lignin is present in greater quantities, as indicated by microchemical stains and the chemical analysis, namely in the vessel walls, wood parenchyma, ray cells, and the lamellae of all cells, *Polyporus gilvus* does not cause delignification, at least to any measurable degree. That is, the decay of wood attacked by *Polyporus gilvus* consists mainly in the destruction of the cellulose, and the highly lignified elements remain in the final stage of decay. Hence this fungus should not be classed among the fungi causing the delignification of wood, but rather placed with those in which "a bleaching action takes place as the rot develops and the typical reaction wherein the lignin compound is removed seems lacking" (22, 1924).

Parasitism

At present there is some confusion among forest pathologists as to the definition of a strictly parasitic wood-destroying fungus. Some investigators would class as parasites those fungi found upon living hosts, others only those found upon living tissue of such hosts. Von Schrenk and Spaulding (44) consider as parasites "such as grow in the living parts of the tree, including the leaves, the younger branches, the newly formed wood and bark, and the

[1] From an unpublished report by the author on the decay of *Quercus rubra* L. caused by *Polyporus gilvus*.

living portion of the root system." There are others who feel that a parasite is an organism which obtains its food directly from living cells. The definition given by von Schrenk and Spaulding would hardly meet with the requirements of this last definition, since not all of the elements of the newly formed wood and bark are alive. Before an annual ring is complete in most woods a large proportion of the cells have already died, for instance, the tracheids, fibers, and cells constituting the vessels. Therefore, if a parasitic fungus is one which feeds upon living tissue, when determining whether a wood-destroying fungus is parasitic or not, it is essential to know what cells are alive within the tissue attacked and whether the fungus invades such cells.

In the sapwood of most broadleaf species the only living cells are the parenchyma cells, namely the ray parenchyma and wood parenchyma. These cells remain alive for several rings back of the cambium, not necessarily throughout the depth of the so-called sapwood (the lighter colored wood directly beneath the bark). Hence a sapwood destroying fungus need not necessarily be invading live cells, that is, not be active as a parasite, since the death of such cells may be only incidental to the disintegration of the already dead cells digested by the fungal enzymes.

Sporophores of *Polyporus gilvus* are commonly found upon the trunks of living trees and consequently the fungus is at times spoken of as a parasite. As already stated, a fungus may exist upon a living tree and still not attack living tissue. Whenever *Polyporus gilvus* was found upon a live tree a careful examination always showed it to be growing upon wood which was apparently already dead. A number of times the fungus has been noticed fruiting upon the stub of a broken branch on a live tree. When this occurred the sapwood and possibly the heartwood of the branch was severely rotted, and a heart rot of the main trunk was at times evident. Sporophores have been observed upon the bark of living tree trunks, which seemed to indicate beyond a doubt that this was a positive case of parasitism. In every instance where this was observed, it was found that beneath the bark was an old lightning scar, possibly a healed over frost crack, or mechanical injury, with the sporophores appearing where the wound tissue had not entirely closed. From the trunk of an injured red oak a very thin section was removed in order to measure the extent of decay. The following year new fruiting bodies were produced upon the trunk, showing that the fungus was still alive. The following two years no sporophores were produced. Four years after the first examination, another careful examination was made. No noticeable increase in width or depth of the decayed zone was found, nor could any tissue cultures be obtained from either the decaying wood or the sound wood bordering upon it. Evidently the fungus had died when that part of the sapwood, which was previously killed, had been wholly decayed, without even attacking the heartwood to any appreciable extent. The early formed callus tissue which had partially

overgrown the extremely decayed sapwood was examined under the microscope for the presence of hyphae, but none were found. At either limit of the wound, new wood had been formed for a depth of several annual rings, but a careful examination of this tissue failed to reveal the presence of hyphae in the cells.

A similar study was made upon the white oak previously mentioned in this paper. Block number one (Figure 2) was used for this study since the apparent limit of decay extended across its face. To the unaided eye, this was evident because of the lighter color of the affected wood. Microscopically there were other noticeable features. Aside from the thinner walls of the fibers in the decayed wood and the presence of many hyphae, the limit of decay was marked by a black zone[1] several cells wide, extending back from the cambium for a distance of eight annual rings. This zone consisted of cells occluded with a brown to black deposit and masses of brown hyphae. Just beyond in the apparently sound wood, tyloses were found in many of the smaller vessels. A few hyphae extended into this same area. Tyloses are formed only by living cells and to all appearances these were formed in the smaller vessels in direct response to a stimulus afforded by the invading fungus, since, behind the black zone in the decayed wood tyloses were not present in the small vessels although they were abundant in the large vessels on either side of it. The cells responsible for the formation of the tyloses within the vessel cavities were alive as indicated by the presence of the protoplast. Hyphae were found to be present in the tyloses of the larger vessels two annual rings back from the cambium where they (tyloses) were still in the process of formation. Apparently then the fungus was present within living cells and consequently there is little doubt but that here it functioned as a parasite according to the strictest definition of the term.

If the fungus were a strong parasite, then one would expect it to have advanced for some distance beyond the limits of the lightning scar. The point at which the wound tissue first formed over the lightning scar, and the absence of tyloses in the small vessels back of the occluded cells, indicated very clearly that the advance into the normal wood was exceedingly slow. It must be kept in mind that the fungus had produced sporophores at this level in the tree for three successive years, and that behind the black zone the wood was in the last stages of decay. The callus tissue covering the extremely decayed sapwood was not attacked by the fungus although the area previously occupied by certain elements of the dead inner bark was filled with masses of hyphae. It is safe to conclude from these studies that *Polyporus gilvus* may live as a weak parasite, but usually, when found growing upon a

[1] This is not to be confused with "black lines of decay" commonly associated with such fungi as *Fomes igniarius* (Linn.) Fries, *Polyporus pargamenus* Fries, etc.

living tree, it derives its food entirely from wood elements that have previously died. This does not agree wholly with the conclusions of von Schrenk and Spaulding (44) that *Polyporus gilvus* (along with certain other sapwood destroying fungi) should be considered as a strict saprophyte. It is improbable however, that this fungus would attack healthy sapwood of an uninjured tree.

Just why the fungus should attack dead cells of the sapwood in dead or injured trees, and not readily attack the same dead cells in the sapwood of living trees, where the sapwood is presumably carrying on conduction, or at least where there are some living cells within the sapwood, the author is unable to state. Nevertheless, it is a fact that in both instances the fibers are dead and, while these are ordinarily the first elements destroyed in the process of decay, under normal conditions these are attacked only when the tree is dead or when that portion of the sapwood ceases to function due to some injury, even though the fungus may be present in the heartwood of the tree.

Summary

1. *Polyporus gilvus*, because of its wide distribution throughout the world and its ability to attack a great variety of woods, is responsible for the destruction of large quantities of wood annually. Although commonly found upon living trees it functions mainly as a saprophyte, however, it has been shown to attack living cells. It probably would never attack living tissues of healthy trees. The fungus shows a preference for sapwood, but at times may cause severe heart-rots. Coniferous woods are apparently resistant to decay by this fungus since only two coniferous hosts are mentioned in literature.

2. *Polyporus gilvus* is found upon living hosts only when they have been injured by lightning, fire, frost, or mechanical means whereby dead sapwood is exposed. Since the fungus is not a strong parasite, its effect upon living trees is not serious; however, it may attack the heartwood of such trees and render that wood unfit for commercial use. Logs, bridge timbers, structural supports, fence posts, and fire wood are often destroyed by the fungus. Within the forest it is economically important in reducing slash to a form available as food for other plants.

3. *Polyporus gilvus* has been grown in culture from the germination of a single spore through to the production of sporophores and viable spores. No secondary spores are produced during the life of the fungus. It has been found that in cultures upon agar, basidia which bear five spores on as many distinct sterigmata, are occasionally produced.

4. Sporulation is continuous over varying lengths of time, but periodic as to the number of spores cast. Spores are discharged

in greater quantities at night than during the day. Lack of light has no apparent effect upon this periodic sporulation. Within limits favoring the production of spores, changes in temperature and moisture produced no apparent change upon the periodic nocturnal sporulation.

5. *Polyporus gilvus* is homothallic as shown by the production of viable spores from sporophores produced in monosporus cultures.

6. Single spore cultures upon blocks of wood carried through the fruiting stage and kept until the fungus died (apparently from lack of food) produced examples of extreme decay in the blocks. This decay corresponded in every detail with the decay of similar woods associated with the fungus under natural conditions, proving conclusively that *Polyporus gilvus* is responsible for the white decay commonly associated with it.

7. A method of producing young sporophores and fresh spores at any season of the year is described. The same method has been used successfully with *Trametes suavolens*, suggesting that other fungi may react in the same manner. This would permit of germination studies from fresh spores at the convenience of the investigator.

8. Stratified sporophores of this fungus are commonly collected. The growth of such sporophores has been carefully studied and it was determined that they did not indicate perennial growth of the fruiting body. The new growth is produced from the mycelium within the wood at the base of the sporophore, and not from any live mycelium within the context or tube layer of the previous year's fruiting body.

9. Chemical changes in the wood during the process of decay were tested with microchemical stains in common use. These tests were verified by a chemical analysis of the decayed and normal wood. The change consists mainly in the destruction of the cellulose structures of the cell walls. Delignification takes place only in those elements where lignin is apparently present in small quantities. The highly lignified elements remain in the most extreme decay with no apparent breaking down of the walls, except for the large number of perforations through which the hyphae passed. That there has been some chemical change is evident from the fact that, (*en masse*) in the decayed wood they appear bleached.

10. Hyphae were found to be present in all stages of decay, appearing most abundantly in the final stages. Here they were usually massed within the larger vessels.

This investigation was carried on in the Botanical Laboratory of the New York State College of Forestry under the direction of Dr. L. H. Pennington, to whom the writer is indebted for constant encouragement and advice. The writer also wishes to express his gratitude to Dr. H. P. Brown, Prof. D. M. Benedict, Dr. L. E. Wise, Prof. A. G. Whitney, and others, for their interest, kindly criticism, and help throughout the work.

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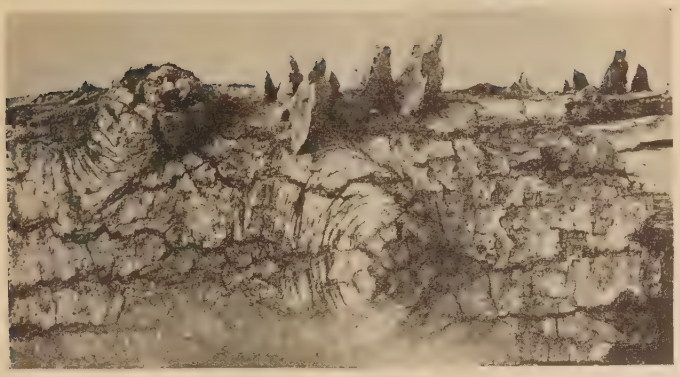

Fig. 1.

Fig. 2.

PLATE I.

Fig. 1. Sporophores of *Polyporus gilvus* (Schw.) Fries on Quercus rubra.

Fig. 2. A stratified sporophore upon *Fraxinus americana*. The older sporophore is not completely covered with the younger growth.

Fig. 3.

Fig. 4.

PLATE II.

Fig. 3. A cross section of a decayed trunk of *Quercus rubra*. The sapwood is completely destroyed and the destruction of the heartwood has begun.

Fig. 4. A cross section taken through the trunk of a living white ash. The decayed area is immediately beneath a fire scar and shows the beginning of a heartwood rot. The darkened line bounding the decayed zone is violet in color when first cut.

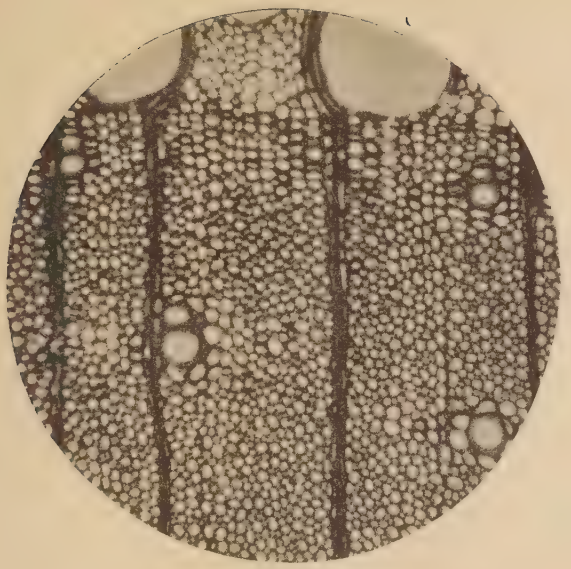

Fig. 5.

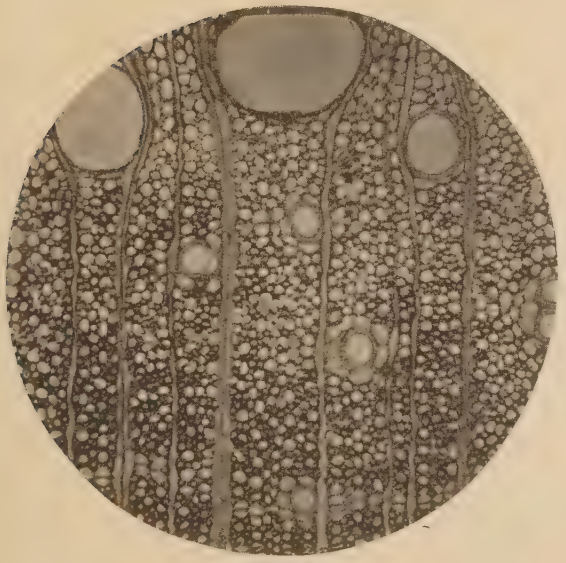

Fig. 6.

PLATE III.

Fig. 5. Normal wood of *Fraxinus americana*.

Fig. 6. Partially decayed wood of *Fraxinus americana*.

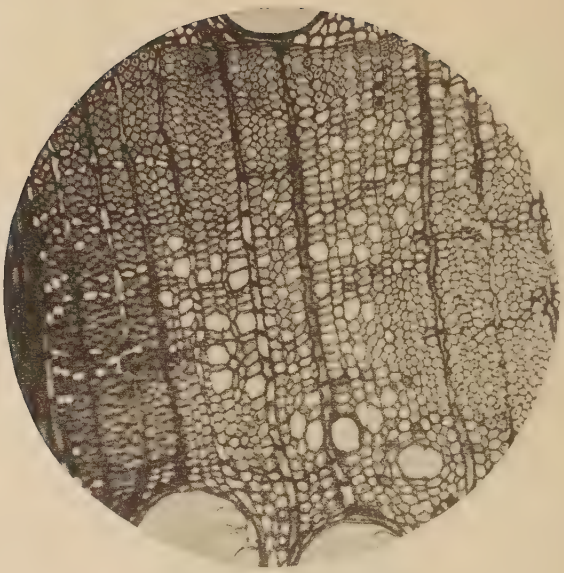

Fig. 7.

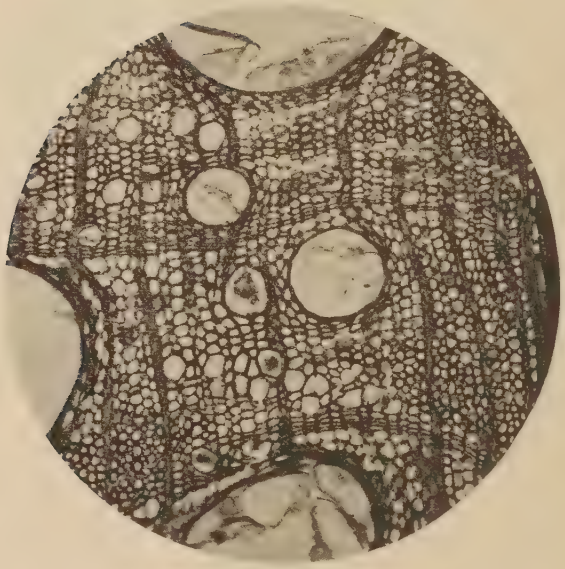

Fig. 8.

PLATE IV.

Fig. 7. Normal wood of *Quercus alba.*

Fig. 8. Decayed wood of *Quercus alba.*

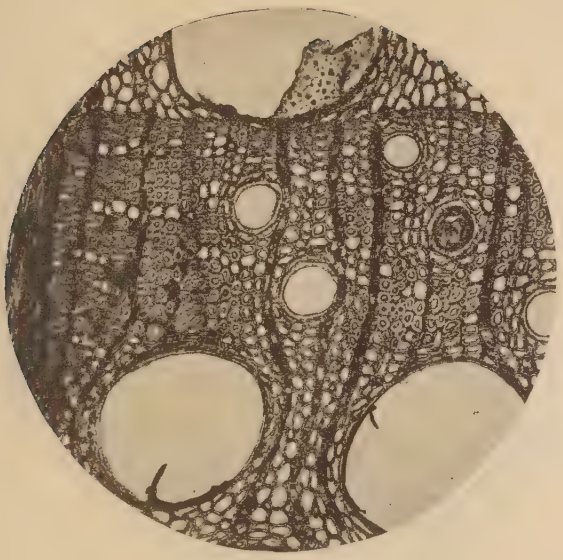

Fig. 9.

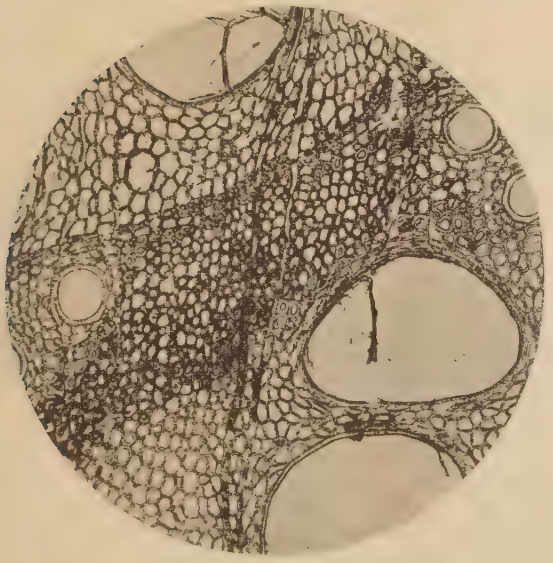

Fig. 10.

PLATE V.

Fig. 9. Normal wood of *Quercus rubra.*

Fig. 10. Incipient decay of *Quercus rubra* showing the fibers to be the first elements attacked.

Fig. 11.

Fig. 12.

PLATE VI.

Fig. 11. Extreme decay of *Quercus rubra* in which only a network of highly lignified tissue remains.

Fig. 12. A cross section taken through the pore surface of a sporophore of *Polyporus gilvus* showing the setae extending beyond the hymenium.

Fig. 13.

Fig. 14.

PLATE VII.

Fig. 13. Fresh spores mounted in water. Note the large globule in each spore.

Fig. 14. A single spore culture of *Polyporus gilvus* upon a maple block. Numerous small sporophores were produced in addition to the larger shelving form.

Fig. 15.

Fig. 16.

PLATE VIII.

Fig. 15. A germinating spore and young primary mycelium.

Fig. 16. A single spore germinated upon agar showing the primary mycelium and the beginning of the secondary mycelium.

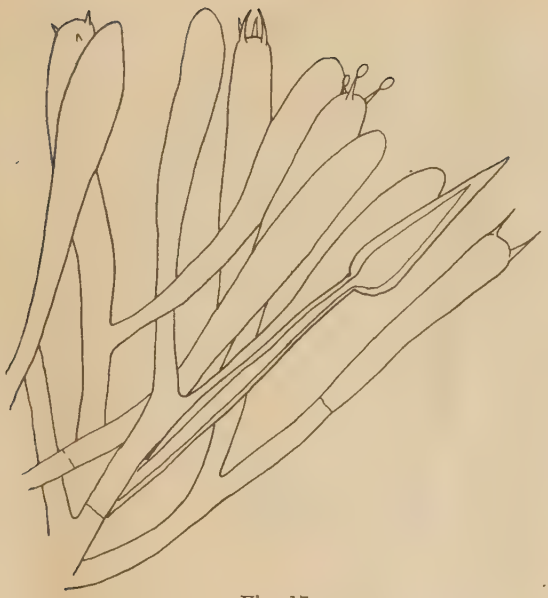

Fig. 17.

Fig. 18.

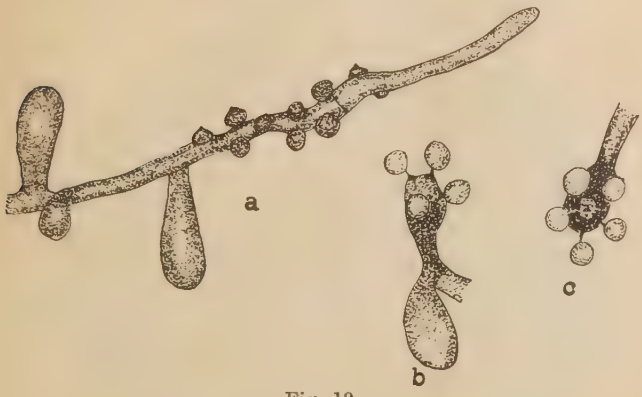

Fig. 19.

PLATE IX.

Fig. 17. A portion of the mycelium from the first formed irpiciform fruiting bodies. Some of the basidia have cast spores, others are immature; one shows basidiospores in the process of development.

Fig. 18. A single basidium with mature basidiospores from poroid fruiting body produced in culture.

Fig. 19. a. Young basidia produced upon mycelium on the surface of agar without the formation of a sporophore. Note crystals on the hypha.

b. A single basidium with four young spores from the same culture.

c. A basidium upon which five young basidiospores were produced. The production of such basidia occurs occasionally when cultures are grown upon the flat upper surface of agar in Petri dishes. If the cultures are turned over, typical poriod sporophores develop at these points.

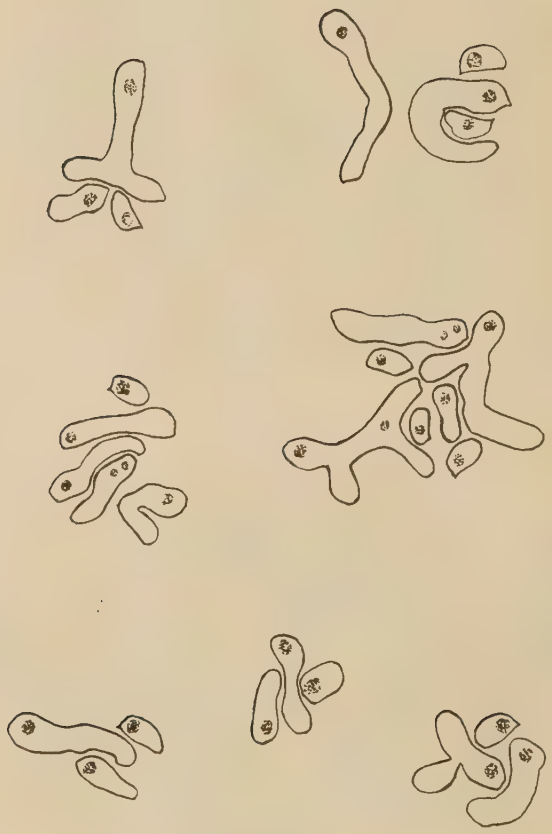

Fig. 20.

PLATE X.

Fig. 20. Basidiospores germinating upon agar. Young germ tubes tend to avoid spores or other germ tubes which usually results in early branching and bending.

Fig. 21.

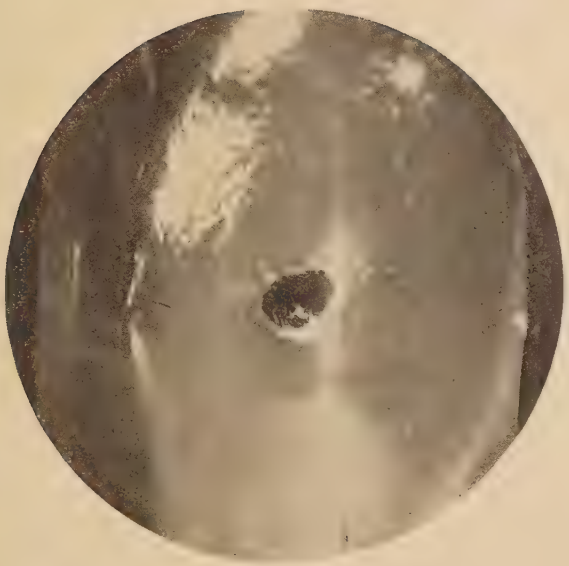

Fig. 22.

PLATE XI.

Fig. 21. An early stage in the formation of a fruiting body in culture showing a mass of crystal-covered hyphae.

Fig. 22. A young fruiting body produced upon an agar slant.

THE NEW YORK STATE COLLEGE OF FORESTRY

SYRACUSE UNIVERSITY